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PHOTOPERIODISM IN RELATION TO HYDROGEN-ION CONCENTRATION OF THE CELL SAP AND THE CARBOHYDRATE CONTENT OF THE PLANT[1]

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INTRODUCTION

In previous papers (*7*, *8*) [2] it has been shown that in many species the duration of the daily period of illumination exercises a remarkable formative action on plant development, and in particular may initiate or inhibit flowering and fruiting. The great majority of species, perhaps all of them, are more or less responsive to differences in the daily light period, though species and varieties differ widely in their sensibility to this influence. In many species the action of the light period under ordinary circumstances is dominant, so that the response of the plant is prompt and certain under a rather wide range of conditions as to temperature, water supply, intensity and composition of the light and the supply of plant nutrients. For example, spring plantings of the four varieties of soy beans (*Soja max* (L.) Piper) known as Mandarin, Peking, Tokyo, and Biloxi, regularly begin flowering in the latitude of Washington about June 15, July 10, August 1, and September 1, respectively, and it has been demonstrated that these differences in time of flowering are characteristic varietal responses to the seasonal change in length of day. Under normal conditions the later varieties are unable to flower until appropriate decrease in length of day has taken place. By artificial shortening of the daily light period all varieties are made to flower at approximately the same time, while, on the other hand, the later varieties may be maintained in the active vegetative condition for a prolonged period of time by continued exposure to a relatively long daily period of illumination (in excess of 13 hours). Artificial light may be effectively used as a supplement to sunlight in prolonging the daily light period. Many other species have been found to be equally responsive to change in the light period, so that in such cases flowering and fruiting may be induced or suppressed at will by proper regulation of the daily illumination period.

Plants which behave like the later varieties of soy beans, that is, tend toward indeterminate increase in stature without flowering when exposed to a relatively long daily light period but quickly flower in response to suitable decrease in the light period, are for convenience designated as short-day plants. There is another large group of plants which tend to remain in the leaf-rosette stage, without stem elongation, and fre-

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[2] Reference is made by number (italic) to "literature cited," p. 155-156.

quently with some form of tuberization when exposed to a short light period, but are capable of developing flowering stems under the influence of a longer daily light period or under certain conditions even when exposed to continuous illumination. Plants of this type are designated as long-day plants.

In both of the above-named groups the rate of growth of the stem increases with increase in the length of the illumination period, maximum growth being associated with sterility in the first group and with flowering and fruiting in the second group. There is a third group, however, in which maximum stem growth is attained with a light period of intermediate length approximating the equatorial length of day. In this group a light period in excess of the optimal for growth favors flowering and fruiting and tuberization. In all cases observed the height of the flower stem, like that of the vegetative stem, increases with increase in the length of the light period, provided the limits favorable to flowering are not exceeded. The bearing of these group relationships on the natural distribution of plants and on the behavior of a given species in different latitudes will be apparent at once to the ecologist. The duration of the light period may induce or modify various other forms of response such as branching habits, root growth, leaf fall and dormancy, but the present discussion will be limited primarily to the fundamental relationships of increase in stature, sexual reproduction and tuberization.

One of the most striking features of the response to duration of the light period so far as concerns initiation of flowering is that the important factor is not merely the total number of hours of illumination during the 24-hour period but the number of hours of uninterrupted light in each period of exposure. Thus, darkening soy beans and other species during the middle of the day for periods of four, five, or six hours, begining at 10 a. m., has but little effect in either hastening or delaying the advent of flowering, though the growth rate may be materially checked.

Another significant fact is that in many cases electric light of intensity as low as 5 foot-candles when used for prolonging the daylight period is capable of exercising a definite formative action in either initiating or inhibiting flowering. It is difficult to explain the action of light of such low intensity on the basis of photosynthesis alone. While the effect on sexual reproduction is clearly expressed, the general nutrition of the plant is not permanently maintained and sooner or later decline sets in, usually resulting in death.

Since sexual reproduction and other forms of expression can be readily controlled by proper regulation of the daily light period this factor furnishes a means for making detailed study of the internal conditions of the plant which are associated with alternative forms of development, at least in so far as analytical methods are available for such work. Under proper conditions and with suitable material the time required for definite expression is easily determined and varies only within narrow limits. Moreover, it is not necessary to control accurately other environmental factors in order to obtain the expected response. It is believed that a large field for profitable study of internal environment in relation to response is thus opened up and the present paper is intended as a beginning in this field. But little is available in the way of a scientific background for such studies, for thus far the duration of the light period as a factor in plant development has received almost no attention from physiologists and biochemists. Even in the case of photosynthesis, while there is a voluminous literature on the effect of intensity and

composition of light, there are no satisfactory data to show whether in any particular species the process proceeds at the same rate and in the same manner with a daily illumination period of, say, 15 hours as with a period of 10 hours. The same is true of transpiration, respiration, and various other plant processes of fundamental importance. The present paper deals with fairly extensive studies of hydrogen-ion concentration of tissue fluids and some preliminary data on carbohydrate content and water relations as affected by the length of the daily light period, and thus associated with alternative forms of expression.

WATER RELATIONS IN PHOTOPERIODISM

Thus far only a beginning has been made in direct experimental study of the influence of the light period on water relations in the plant, but in connection with the other studies discussed in this paper it seems desirable to direct attention to observed plant reactions which suggest a definite relationship between the length of the illumination period and the water content of the plant. That maintenance of maximum turgidity is in some way favored by a light period which is optimal for increase in stature is illustrated by the fact that in certain cases transfer from such light conditions to those which are suboptimum for increase in stature quickly results in a change from the upright toward the horizontal or prostrate position of the stem, although growth may be maintained. In other cases, this change in the light relations causes dying back of the stem, followed by development of new basal shoots. Moreover, increased pubescence may be a feature in the change from an optimal to a suboptimal light period for upward stem growth. These phenomena and other features of development commonly seen under relatively xerophytic conditions are observed when the plants are abundantly supplied with water and even under fairly wide ranges in the external water supply.

It is well known that as a rule exposure to sunlight greatly increases transpiration and it seems possible that in those species for which an intermediate length of day is optimal for growth the check in rate of growth resulting from exposure to long days is due at least in part to excessive transpiration. For other species, however, the rate of increase in stature is more or less proportional to the length of the daylight period and loss of turgidity resulting from decrease in the daily light period would seem to be due to changes in internal conditions of the plant. From the investigations of Briggs and Shantz (*4*, *5*) and Livingston (*14*), together with the work of earlier investigators, it might be assumed that in the first case the excessive transpiration (if such actually occurs) is probably due chiefly to external evaporative forces. In those species in which maximum turgidity is maintained by exposure to a long daily illumination period internal factors are apparently involved, for it is not clear as to how reduction in the period of illumination could through direct action cause decrease in turgidity as a result of excessive transpiration. According to Livingston and Brown (*15*), exposure to sunlight may result in incipient drying of the foliage leaves, which in turn may tend to check transpiration to some extent by causing partial closure of the stomata and decreased partial pressure of water vapor in the leaf. There seems to be no ground at present, however, for supposing that increased duration of the light period would tend to accentuate the effectiveness of these factors in checking transpiration to the point of overcoming external evaporative forces. Changes in osmotic relations,

permeability of the protoplasmic layer and the hydration capacity of the hydrophilic colloids, might be regarded as possible factors in the reduced water-holding capacity of the plant tissues when the plant is exposed to illumination periods which are too short for maximum rate of increase in stature.

Prompt initiation of sexual reproduction when certain species are exposed to a reduced daily illumination period is perhaps the most striking phenomenon of photoperiodism, and in the case of the violet, at least, this response has been shown to be associated with partial loss of turgor. Here again is seen a possible relationship with the well-known fact that flowering and fruiting are favored by conditions of comparative drought, as has been especially emphasized by Möbius (*20*). That darkening species of this type for several hours in the middle of the day fails to initiate flowering possibly may be due in part to resultant check in rate of transpiration, thus enabling the plant to maintain higher turgidity. There is the possibility that the reduced rate of growth under these conditions is explainable on the basis of reduced photosynthesis.

There can be no doubt that the water relations of the plant are profoundly affected by the duration of the daily illumination period, but it is impossible to state whether change in water content stands in a direct causal relation to observed responses of the plant or merely follows as a sequel to other internal processes more directly controlled by the light period.

ACIDITY RELATIONS IN PHOTOPERIODISM

The theory advanced by Liebig (*13*) that organic acids are intermediate products in the photosynthesis of carbohydrate has not met with favor among plant physiologists and it has come to be the generally accepted view that these acids result from partial oxidation of carbohydrate and fat. While formation of organic acids thus seems to be more directly related to respiratory activity than to photosynthesis, there is no doubt of the fact that light is in some way a dominant factor in the origin of these catabolic products, as will be developed more fully in the present discussion. Some of the acids formed are neutralized by mineral bases derived from the soil, and to the extent that insoluble salts are formed these tend to accumulate in the plant tissues. According to de Vries (*25*), however, the total quantity of acids formed in a period of 100 days may greatly exceed the total dry weight of the leaf, so that obviously the greater portion of the acids formed must undergo decomposition, the final products being carbon dioxide and water. It is generally believed that light is a very important factor in the breaking down of organic acids in the plant cell. It is apparent that the total acidity at any particular moment is dependent, on the one hand, on the rate of formation of acids and, on the other hand, on the rate of their decomposition and, to some extent, perhaps, on the quantity neutralized by bases derived from the soil. In addition to light, temperature has been found to be one of the important external factors influencing relative rate of formation and decomposition of acid, high temperatures favoring net decrease in acidity.

With respect to comparative total acidity of different organs of the plant and of particular organs at different stages of development the most extensive previous observations are those of Astruc (*1*) who investigated the total free acid content of a number of species. This inves-

tigator, however, failed to recognize the effect of alternative forms of activity on the distribution of acidity in the different parts of the plant, so that his results as a whole can not be readily interpreted. In brief, he found that in the leaf the acidity decreases with increasing age of the organ, in the stem the acidity decreases from the apex downward, while in the floral organs acidity decreases from the button stage up to unfolding of the blossom, and the developing fruit also shows decreased acidity.

Various functions in the economy of the plant have been ascribed to organic acids, but detailed discussion of the different theories which have been advanced on the subject will not be here undertaken. It was long ago suggested that the free acids and their soluble salts play an important rôle in promoting osmotic pressure, turgor, and growth, and this theory in its relation to change in the acidity of plants when exposed alternately to light and to darkness has been widely discussed (cf. Kraus (*12*)). This is the most important of the older theories regarding the effect of acid content of the plant fluids on metabolism, and the general procedure which has been followed in studying the subject involves measurement of total acidity by titration. In the application of more recent conceptions regarding the effect of acidity on the properties of plant colloids, however, it becomes necessary to give special consideration to relations of active acidity[3] of the tissue fluids, as measured by concentration of hydrogen ions. The latter phase of plant acidity has not thus far been fully developed. According to Atkins (*2*) plant cells rarely show an alkaline reaction and the P_H value of the sap does not exceed the number 8. On the other hand, in certain fruits the P_H value of the sap may fall as low as 1.4. According to this author "the P_H value met with in a tissue is usually near, but slightly less than, the optimum for the activity of the characteristic enzyme at ordinary temperature." Clevenger (*6*) studied the range in hydrogen-ion concentration in leaf, stem and root of cowpeas during the 24-hour period, and found the highest concentration in leaf and stem to occur during the morning hours, while the lowest concentration was observed during the night. The root showed a narrower daily range in acidity and the maximum occurred during the day. Of the three plant parts the stem averaged highest and the root lowest in active acidity. From observations on several crop plants Haas (*10*) finds that the active acidity of the sap is affected by changes in illumination, the soil solution, the age of the plant, and other conditions. Several publications have appeared in recent years which deal with the hydrogen-ion concentration of the cell sap as affected by the reaction of the soil solution or the culture medium, but these problems are not directly involved in the present discussion and hence may be passed over.

The significance of measuring hydrogen-ion concentration in studying the relation of acidity to the properties of colloidal proteins has been especially emphasized by Loeb (*16*). In view of the effects of acidity, as thus measured, on the swelling of gels and on the viscosity and osmotic pressure of protein solutions, it is desirable to obtain much more complete information than has hitherto been available as to the influence of external environment on the hydrogen-ion concentration of the cell fluids. It is hoped that the present contribution will add materially to

[3] The term "active acidity" refers to the portion of any acid substance which has become ionized and thereby rendered active in producing the effects commonly ascribed to acids; it is measured in terms of hydrogen-ion concentration.

the available data on the effect of light, and more specifically the duration of the daily illumination period, on the active acidity of the cell sap.

This problem is complicated by the marked differences in acidity which are found in the different organs and tissues of the plant under varying environmental conditions. An additional complication is the progressive change in acidity relations with increase in age of the individual organs and of the plant as a whole. In this connection, it is necessary also to keep in mind the fact that the "age" of the organ or organism, as indicated by the stage of development attained, can not be measured solely by lapse of time but is dependent largely on the particular environment to which the plant happens to be exposed. To give a concrete illustration, when late-flowering cosmos is exposed to a daily illumination period of, say, 15 hours, the apex of the stem shows a progressive and marked increase in hydrogen-ion concentration as growth proceeds, maximum acidity being attained only after the lapse of several weeks. In this case vegetative development of the stem continues for a more or less indefinite period. On the other hand, if exposed to 10 hours of light daily, the apex of the stem shows no such marked increase in active acidity until after the flower bud has appeared. Under the longer illumination period the active acidity of the apex is much greater than that of the base of the stem, while under the shorter illumination period these relations may be actually reversed. Proper sampling of the material for study offers considerable difficulty, since for practical purposes it is often impossible to separate completely tissues, or organs, even, especially in the earlier stages of development. In most cases, however, the differences in acidity are so well defined as to leave no doubt as to the adequacy of the methods of sampling followed, where only comparative results are required.

In these experiments, except in special cases, no effort was made to follow out in detail the change in acidity through the 24 hours of the day, for such a procedure is out of the question where it is necessary to conduct a large number of separate tests. This difficulty was overcome as far as possible by choosing different hours of the day for making the observations in the course of a particular series, always collecting samples at as nearly the same hour as possible in comparing material exposed to two different illumination periods. In certain cases, moreover, comparative readings were made in the morning and afternoon hours, as a further check on the results obtained. In all cases the hour at which sampling was done was recorded and the material was used for observation as soon as possible after it had been collected.

In carrying out the measurements the plant material was crushed in a porcelain mortar or in a tinned food chopper and the crushed material was strained through several thicknesses of cheesecloth. The hydrogen-ion concentration was determined electrometrically, the potential being measured with either a Northrup "millivolter" or a Northrup hydrogen-ion "pyrovolter" and a Leeds and Northrup small, portable galvanometer. The saturated KCl-Calomel electrode was used and the hydrogen electrode was made and "platinized" essentially as directed by Bovie (*3*). The platinum coat on the wire, however, was always deposited just before being used. Electrolytic hydrogen compressed in cylinders was caused to bubble through the sample of sap and the potential was first observed after a period of 10 minutes. Further observations were made at intervals of 5 to 10 minutes until a constant reading was obtained,

this usually requiring 10 to 20 minutes. The temperature of the air was noted and the P_H value [4] of the sample calculated. The accuracy of the outfit used was frequently checked by observing the potential of a standard acetate solution or of a solution of potassium acid phthalate.

DAILY PERIODICITY IN ACIDITY OF THE CELL SAP

Before passing to detailed consideration of the effect of differences in the light period on the average level of plant acidity it is necessary to discuss briefly the subject of the daily change in acidity during the 24-hour period, involving both the formation and the decomposition of acid. That there is a rhythmic daily change in acidity in succulents has been known since [1813], when Heyne (*11*) made the observation that leaves of *Bryophyllum calycinum* possess a more acid taste in the early morning than in the afternoon. Quantitative observations on the subject were made by A. F. Mayer (*17*) in 1875, and he confirmed the fact that in succulents there is an increase in acidity at night and a decrease during the day. Similar periodicity, however, could not be detected in Oxalis species. Mayer found that in the absence of CO_2 Oxalis gives off no oxygen when exposed to sunlight, and therefore concluded that organic acids are not intermediate products of photosynthesis. Bryophyllum, on the other hand, does evolve oxygen gas in sunlight in the absence of an external supply of CO_2. Increase in temperature from 20° to 30° C. causes a decrease in acid content in Oxalis, which was ascribed to increased respiratory activity. In later publications (*18*, *19*,) Mayer undertakes to show that the evolution of oxygen by succulents when exposed to sunlight in an atmosphere free from CO_2 is due to splitting of oxygen from the CO_2 which is formed by the plant and again utilized in photosynthesis. G. Kraus (*12*) studied various phases of acidity relations in plants. He observed a decrease in acidity in several species of the nonsucculent type when exposed to sunlight and, as a result, made the mistake of assuming that the phenomenon of increase in acidity at night and decrease during the day applies quite generally to nonsucculents as well as to succulents. Kraus found that deacidification is due to direct action of light and is not dependent on either respiration or photosynthesis. This investigator concluded that the content of mineral salts of organic acids in the tissues does not change materially from day to night or from day to day, hence daily periodicity in acidity is not due to the neutralizing action of bases derived from the soil.

In an important contribution to the subject of plant acidity de Vries (*25*) brought out several facts of special significance in their bearing on the present discussion. The marked increase in acidity in succulents occurring at night is due to previous action of sunlight, for the nocturnal rise in acidity does not occur in the absence of illumination by day. Moreover, in continued darkness there is progressive decrease in acidity during both day and night after the first 24 hours, and this phenomenon seems to apply to thin-leaved species as well as to fleshy plants. The action of sunlight in causing subsequent rise in acidity is not due to a heat effect, for warming in darkness during the day does not cause increased acidity at night. Also, the action of light is not due directly or solely to photosynthesis, for exposure to light in an atmosphere free from carbon dioxide

[4] It may be pointed out here that the P_H value represents the potential due to hydrogen ion in a solution, and is the negative logarithm of the actual concentration of this ion. Hence, the higher the acidity, the smaller the P_H number; and a change of one P_H unit means a tenfold change in hydrogen-ion concentration.

causes rise in acidity at night. Very weak light which could not cause marked photosynthetic activity is able to promote nightly rise in acidity. A very short period of illumination, on the other hand, causes no rise in acidity at night. The increase in acidity at night is due to a greater rate of formation than of the destruction of acid, while under high temperatures the rate of destruction may keep pace with the rate of formation. De Vries concludes that accumulation of acids at night is largely limited to fleshy plants. Finally, light action is not the cause of deacidification, but merely promotes the process. Warburg (*26*) in his paper on the subject gives a thorough review of previous work, and records observations on a large number of species. This author finds that decrease in acidity in the presence of sunlight does not take place in all thin-leaved species. In general, the daily decrease in acidity is proportional to the degree of protection against transpiration afforded by the structure of the plant organ. Plant parts which are free from chlorophyll show little or no decrease of acidity in sunlight. In the green parts of succulents deacidification both in the presence or the absence of light is coupled with the presence of oxygen. Increase in acidity at night is probably dependent on increase in sugar content which results from illumination during the day. The presence of oxygen, though in only small quantity, is required for acid formation. The acids of succulents are to be regarded simply as products of incomplete oxidation. The formation of acids in plant tissues is proportional to (1) the intensity of metabolism, (2) the degree of protection against entrance of oxygen; the decomposition of acids is proportional to (1) the intensity of metabolism, (2) the accessibility of atmospheric oxygen, (3) the temperature.

Puriévich (*22*) finds that decrease in acidity of leaves in prolonged darkness is not due to physiological translocation or to neutralization of the acid by bases derived from the soil. The formation of acid in the dark is proportional to the intensity and duration of the preceding illumination and leaves placed in sugar solutions showed increased formation of acid. Transfer from the light to darkness causes a gain in acidity for a period ranging from 8 hours in some species to more than 24 hours in other species, and these differences are believed to be correlated with differences in the relative stability of malic, oxalic, tartaric, and citric acids, since different species do not contain the same acids. In the spontaneous decomposition of solutions of these acids *in vitro* when exposed to sunlight malic acid is most easily broken down, followed by tartaric and oxalic, respectively, while citric acid is not affected. Oxalic and malic acids, however, are most affected by increase in temperature. In a comparatively recent review and study of acidity in succulents, with special reference to cacti, Richards (*23*) finds maximum acidity of the plant juice at about 7 o'clock a. m. and the minimum at about 5 p. m., in the case of *Opuntia versicolor*. The greatest daily range in acidity occurs in summer. The daily range was greater in younger joints of the plant than in older ones. The diurnal change in acidity is due mainly to sunlight, while temperature also is a factor. The formation of acid in the plant is due to an inadequate supply of oxygen in the tissues. Deacidification is not believed to be a part of the respiratory process. As bearing on the work of Puriévich relating to the relative rate of decomposition of different acids in sunlight and the more recent study of the subject by Spoehr (*24*), attention is called to the fact that the same difference in ease of decomposition between malic and citric acids applies to deacidification in darkness, as has been

shown in this office in the case of tobacco leaves during the process of curing (*9*). During the progress of the curing there is a decided decrease in total content of malic acid, while there is an equally marked increase in content of citric acid.

From the above-mentioned investigations it appears that clearly defined daily periodicity in acid content, with marked decrease in acidity during the daylight period and a corresponding increase in acidity at night, is characteristic of succulents having structural features which are unfavorable for rapid gas exchange. This periodicity is much less evident in thin-leaved species and in some of these has not been observed to occur at all. Formation of acids in plant tissues results from incomplete oxidation of carbohydrate and is favored by a limited oxygen supply. While, in the case of succulents, the acids accumulate chiefly during the night, nevertheless the rate of acid formation is in some way dependent primarily on the conditions of illumination during the day. Decomposition of acids in the tissues of the plant is facilitated by increased oxygen supply, by exposure to light, and by relatively high temperatures. Exposure to light, however, is not a necessary condition for acid decomposition. There seems to be considerable doubt, moreover, as to whether deacidification is to be regarded as a part of the respiratory process.

The present investigations, relating primarily to the average level of acidity of the plant sap as affected by the duration of the light period, have incidentally emphasized the relatively narrow daily range in acidity of the thin-leaved species which have been studied. Direct observations also have been made on this point, in the case of Biloxi soy beans. A series of readings were obtained on the sap of the young, topmost leaves of the plants collected early in the morning, at noon, and late in the afternoon. In plantings made in the field the average P_H values of the saps during the midsummer, when the plants were most active vegetatively, were 6.22, 6.19 and 6.14, respectively, for the samples collected in the morning, at midday and in the afternoon. Similar readings taken later in the season, when the plants were approaching or had actually reached the flowering stage, showed practically no change in active acidity during the day. These results were confirmed by a second series of observations on plantings made in boxes and exposed to the natural length of day of early and late summer. Finally, in plantings in boxes exposed to only 10 hours of light daily, a light period which, of course, quickly initiates flowering, there was at most only a slight increase in acidity during the day. Thus, in contrast with the behavior of succulents, Biloxi soy beans show an appreciable increase in hydrogen-ion concentration of the sap from morning to afternoon as long as the plants continue in the active vegetative stage as a result of exposure to relatively long days. When the flowering stage is reached as a result of exposure to relatively short days there is little or no daily change in active acidity.

In plants which show the behavior of succulents it might be expected, perhaps, that an increase in the number of hours of daily illumination would tend to reduce the average acidity for the 24-hour period. In any event, with respect to thin-leaved species it may be stated that in the limited number observed in the present investigation the general tendency has been toward an increase in average active acidity with increase in duration of the daily light period. This holds true in general both

for plants which flower when exposed to relatively short days and for those which flower when exposed to long days. In detail, however, there are important differences in the actual course of the acidity level during growth and development in the two classes of plants, for changes in form of development resulting from differences in the light period are associated with definite changes in average acidity of the cell sap. Moreover, decrease in the duration of the light period to which the plant is exposed may not result in immediate decrease in average active acidity. Apparently, the full effect of such change in illumination is not seen, as a rule, till about the third to fifth day, thus suggesting similarity to the behavior of succulents in continued darkness.

EFFECT OF THE LIGHT PERIOD ON ACIDITY RELATIONS IN SHORT-DAY PLANTS

In the study of acidity of the cell sap as affected by the duration of the illumination period four general forms or features of plant development have been included, namely, (1) the sterile vegetative stem; (2) stem

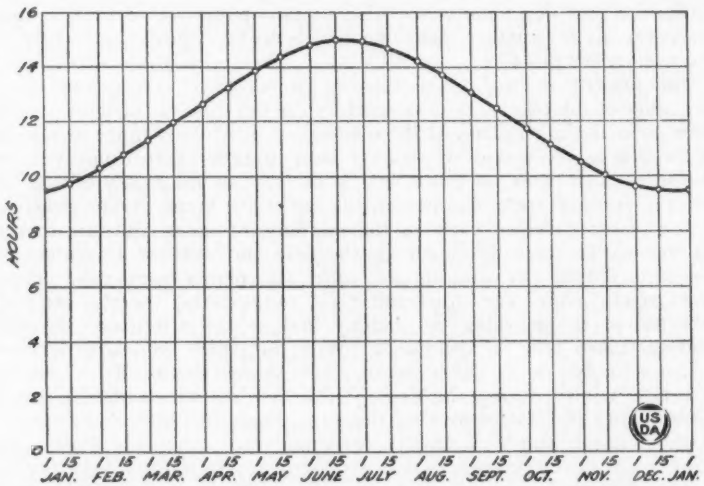

FIG. 1.—Approximate length of day, from sunrise to sunset, for the latitude of Washington, D. C.

elongation accompanied by flowering; (3) the leaf-rosette type of growth, with clearly defined tuberization; (4) the leaf-rosette type of growth, without pronounced tuberization. For comparative purposes the plants may be grown throughout their development under the respective light periods favorable to these different types of activity or at the proper time the plants may be shifted from one light period to another so as to change the type of development. Both plans have been followed. To facilitate comparison of the artificially regulated daily light periods employed in the experiments with the prevailing natural length of day (sunrise to sunset), the approximate annual range in day length in the latitude of Washington is shown in figure 1.

As already explained, the term "short-day plants" is applied to those species and varieties which tend to produce indeterminate vegetative

stems when exposed to long days but quickly flower when exposed to short days. Late-flowering varieties of *Cosmos bipinnatus* Cav., Biloxi soy beans, the Maryland Mammoth variety of tobacco (*Nicotiana tabacum* L.), and *Tithonia rotundifolia* (Mill) Blake are examples of this group. In this type of plant the height attained is usually proportional to the duration of the daily light period, whether or not flowering occurs. In most cases, however, it is not possible to completely eliminate stem elongation by shortening the light period, so that only the two first-named forms of development need be considered. A rather extreme illustration of these two contrasted forms of development in *Bidens frondosa* L., which behaves in the same manner as *Cosmos bipinnatus*, is shown in plate 1, A.

EXPERIMENTS WITH COSMOS BIPINNATUS

Late-flowering white cosmos was planted in boxes on May 2 and had germinated on May 6. One lot of plants received only 10 hours of illumination daily from the beginning of the experiment, a second lot was exposed to the natural length of day up to and including July 11 and thereafter was exposed to 10 hours of illumination daily, while a third lot was exposed to the natural length of day throughout the test. Very small flower buds could be seen on the plants of the first lot by June 1 and on the second lot by July 24, while no flower buds were visible on the third lot of plants till September 19. The hydrogen-ion concentration of the cell sap of the apex and the topmost and basal portions of the stem under the two conditions of illumination is shown in Table I. The material designated as "apex" includes the growing point, the extreme tip of the stem and the attached leaflets. For "top of stem" a section 1½ to 2 inches long immediately below the apex was used and a section immediately above the ground level of the same length was employed as "base of stem." The data presented in this table include observations at fairly wide intervals for the plants exposed to a 10-hour day from the outset and for the earlier stages of development of the plants exposed to the natural length of day. More frequent observations are given for the plants transferred from the natural length of day to the 10-hour day after considerable growth had already been attained and for later stages of development of the plants under the full length of day of summer. In order to complete the data, a second series of observations was made in such way as to supplement the data of Table I. For this purpose a second planting of the white cosmos was made on June 5 and the seed had germinated on June 8. One series was grown from the beginning under a 10-hour day and a second series was exposed to the full length of day of summer throughout the test. Under the shortened length of day flower buds were found on June 30, but none appeared during the test under the long-day conditions. The observations were made in the same manner as before, and the results are shown in Table II.

TABLE I.—*Showing the P_H value of the cell sap of the apex and upper and lower portions of the stem of late-flowering white cosmos under the natural length of day and under a 10-hour day during the spring and summer months (seed germinated May 6)*

Plants exposed to natural length of day.							Plants exposed to a 10-hour day.[a]						
Date of sample.	Hour of sampling.			P_H value.			Date of sample.	Hour of sampling.			P_H value.		
	Apex.	Top of stem.	Base of stem.	Apex.	Top of stem.	Base of stem.		Apex.	Top of stem.	Base of stem.	Apex.	Top of stem.	Base of stem.
May 19	11.30 a. m.	12.30 p. m.	12.30 p. m.	6.24	5.82	5.60	May 20	12.05 p. m.	1.10 p. m.	1.10 p. m.	6.36	5.70	5.69
June 2	12 noon	12.55 p. m.	2.20 p. m.	6.12	6.28	5.67	June 1	11.45 a. m.	12.45 p. m.	1.25 p. m.	6.29	6.17	5.74
June 9	12.30 p. m.	1.10 p. m.	2 p. m.	5.84	5.80	5.74	June 8	11.55 a. m.	1.25 p. m.	3.10 p. m.	[b] 6.05	[c] 5.63	5.63
July 1	9.55 a. m.	10.30 a. m.	11.15 a. m.	5.33	5.37	5.34	June 15	12 noon	1 p. m.	1.55 p. m.	[b] 5.95	[c] 5.64	5.71
July 12	12 noon	12.50 p. m.	1.30 p. m.	5.23	5.24	5.40	July 14	10.05 a. m.	12.30 p. m.	2.30 p. m.	5.33	5.36	5.41
July 14	9.05 a. m.	11.40 a. m.	1.20 p. m.	5.36	5.43	5.33	July 17	9.45 a. m.	12.20 p. m.	2.15 p. m.	5.53	5.33	5.32
July 17	8.50 a. m.	....do.	1.15 p. m.	5.20	5.35	5.16	July 19	10.20 a. m.	12.40 p. m.	2.40 p. m.	5.48	5.54	5.36
July 19	9.20 a. m.	11.55 a. m.	1.45 p. m.	5.25	5.38	5.36	July 21	12.20 p. m.	1.15 p. m.	2.25 p. m.	5.53	5.46	5.29
July 24	8.40 a. m.	10.25 a. m.	1.25 p. m.	5.30	5.25	5.35	July 24	9.30 a. m.	12.15 p. m.	3 p. m.	5.54	5.33	5.39
Aug. 5	12.40 p. m.	1.30 p. m.	2.30 p. m.	5.16	5.31	5.32	Aug. 1	12.20 p. m.	1.20 p. m.	2.30 p. m.	[b] 5.99	[c] 5.68	5.46
							Aug. 11	8.55 a. m.	8.55 a. m.	.....	[d] 5.27	[e] 5.43	.....

[a] The data for the period May 20 to June 15, inclusive, relate to plants exposed to the 10-hour day from the beginning of the experiment; the data for the subsequent period relate to plants exposed to the natural length of day up to and including July 11, and thereafter exposed to the 10-hour day.
[b] Flower buds, only.
[c] Topmost section of flower bud stem.
[d] Newly opened blossoms.
[e] Topmost section of flowering stem.

TABLE II.—*Showing the P_H value of the cell sap of the apex and the upper and lower portions of the stem of late-flowering white cosmos when grown from germination (June 8) under the natural length of day and under a 10-hour day during the summer months*

Date of sample.	Plants exposed to natural length of day.						Date of sample.	Plants exposed to a 10-hour day.					
	Hour of sampling.			P_H value.				Hour of sampling.			P_H value.		
	Apex.	Top of stem.	Base of stem.	Apex.	Top of stem.	Base of stem.		Apex.	Top of stem.	Base of stem.	Apex.	Top of stem.	Base of stem.
June 19	11.45 a. m.	12.35 p. m.	12.35 p. m.	6.46	5.80	5.78	June 20	12 noon	1 p. m.	1 p. m.	6.26	5.91	5.64
June 22	12.30 p. m.	1.35 p. m.	1.35 p. m.	6.52	6.17	5.79	June 23	12.50 p. m.	1.50 p. m.	1.50 p. m.	6.46	5.82	5.69
June 26	11.45 a. m.	12.40 p. m.	12.40 p. m.	6.46	5.94	5.56	June 27	11.45 a. m.	12.45 p. m.	12.45 p. m.	6.24	5.85	5.66
June 29	12.35 p. m.	1.40 p. m.	1.40 p. m.	6.36	6.09	5.65	June 30	12.05 p. m.	1.10 p. m.	1.10 p. m.	6.22	5.86	5.53
July 6	11.40 a. m.	12.35 p. m.	12.35 p. m.	6.18	6.17	5.65	July 3	12.25 p. m.	1.25 p. m.	1.25 p. m.	[a] 6.17	5.76	5.57
July 20	12.05 p. m.	12.55 p. m.	1.55 p. m.	5.59	5.69	5.65	July 10	12.15 p. m.	1.15 p. m.	2.40 p. m.	[b] 6.06	5.67	5.59
Aug. 8	8.40 a. m.	9.30 a. m.	11.45 a. m.	5.27	5.42	5.49	July 13	12.40 p. m.	1.30 p. m.		[b] 5.82	5.62	
							July 27	12.15 p. m.	12.15 p. m.	1.50 p. m.	[c] 5.26	[d] 5.48	5.57
							July 28	9.30 a. m.			[e] 5.58		

[a] Small flower buds rejected in sampling.
[b] Flower buds only.
[c] Fully opened blossoms.
[d] Topmost section of flower stems.
[e] Seed heads, in green stage.

The results recorded in Tables I and II, taken as a whole, are quite consistent and leave no doubt as to the conclusions to be drawn. There is a striking difference in the course of the acidity relations during the progress of development of the plant under the long-day and short-day conditions and these differences are clearly associated with differences in form of expression caused by the two conditions of illumination. The seat of maximum change in acidity is found in the region of the growing point. Under the longer daily illumination period the plants at the age of 2 to 3 weeks show a relatively low acidity, particularly in the apical structures, but with continued elongation of the stem there is a marked, progressive increase in acidity, the P_H number for the apex declining from a value well above 6 to approximately 5.2, at which point it remains comparatively stationary (fig. 2). The advanced stage of vegetative development of the stem, therefore, is characterized by relatively high acidity of the apex as compared with early stages of develpment. The

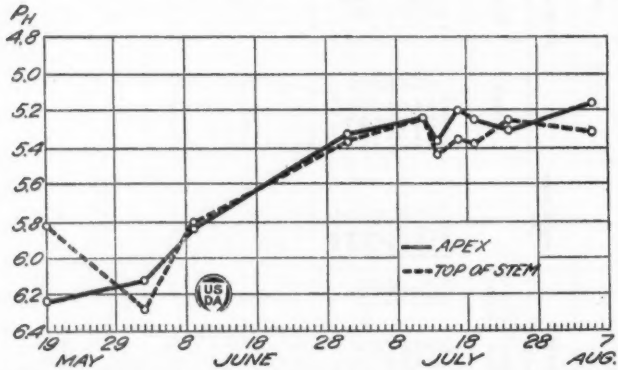

FIG. 2.—Showing the characteristic progressive increase in active acidity in the sap of the apical tissues of late-flowering cosmos when exposed to the long days of summer (Table I). Cosmos is a typical short-day plant and in this group indeterminate elongation of the vegetative stem under a long-day exposure is associated with a progressive increase in active acidity in the region of the apex. The relatively high active acidity of the advanced vegetative stage is maintained during the period of active vegetative growth.

portion of the stem immediately below the apex shows greater acidity than the apex in earlier stages of development, but as growth proceeds this relationship is reversed. The base of the stem is initially the most acid portion of the plant, but shows only moderate subsequent change, and hence in later stages of growth tends to become the least acid part of the plant (fig. 3).

When the plant is exposed to a 10-hour day from date of germination, which has the effect of promptly initiating flowering, there is an appreciable but transitory decrease in hydrogen-ion concentration in the tissue fluids of the apex, which takes place about 2 weeks after germination. The probable significance of this phenomenon is later referred to more fully(p. 148.) This temporary decrease is followed by increase in acidity till the P_H number of approximately 6 is reached, a level of acidity which is characteristic of the flowering condition and represents the acidity of the young flower buds themselves. The flower buds and adjacent tissues increase in acidity till the unfolding of the blossom is completed. This latter stage marks the culmination of the increase in acidity of the repro-

ductive structure, and it is interesting to note that the P_H value is the same as that finally attained in the apex in advanced vegetative development (fig. 4). There is but little change in acidity in upper and lower portions of the stem except that after flowering has been initiated the former increases somewhat in acidity. As compared with the action of long days, the effect of the 10-hour day on the plant during the first 3 weeks of growth following germination is to increase the acidity of the cell sap, except for the above-mentioned temporary decrease in acidity under the short-day exposure.

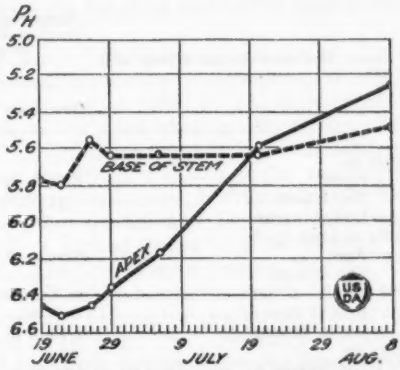

FIG. 3.—Showing the active-acidity relations in the apex and base of the stem in late-flowering cosmos when exposed to the long days of summer (Table II). In contrast with the sharp progressive increase in active acidity of the apex in this group of plants when exposed to long days there is but little change in the active acidity of the base of the stem. As a result, advance in vegetative development leads to a reversal in relative acidity of the apex and basal portion of the stem.

After the acidity relations characteristic of the advanced stage of vegetative development have become established abrupt change from long-day to short-day conditions results in decided decrease in acidity of the apex by the fifth day, but this change is not apparent by the second day after the transfer had been made (fig. 5). Comparing acidity relations under the short-day exposure with those under the long-day exposure, it is evident that at the time the flowering condition is attained under the former the acidity gradient of the axis is the reverse of that found in the advanced stage of vegetative development under the latter exposure, but is similar to the gradient found in the earlier stages of growth under the long-day exposure. As a matter of fact, the plants exposed to the longer daily illumination period show at a certain stage acidity relations quite similar to those characteristic of the flowering condition, as shown in the data of July 6 in Table II. On the other hand, at the moment the unfolding of the blossom is completed the acidity relations are quite similar to those seen in the advanced stage of vegetative development.

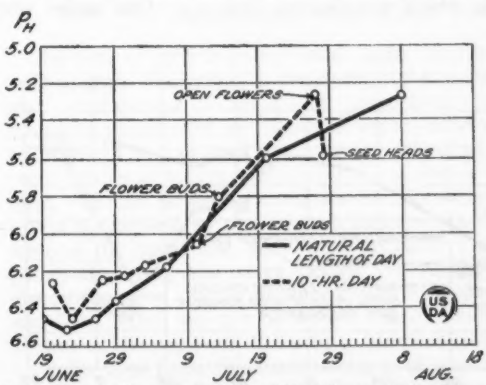

FIG. 4.—Showing the course in active acidity of the apex in late-flowering cosmos when exposed to the relatively long days of summer and to a 10-hour day, respectively, from date of germination (Table II). In short-day plants of this type exposure to a 10-hour day promptly initiates flowering. Under these conditions there is during the early stage of development a well-defined temporary decrease in active acidity (compare fig. 10), probably indicative of initiation of flowering. There is then a progressive increase in acidity, culminating in the freshly open blooms and finally a decrease in the developing seed head or fruit.

TABLE III.—*Showing the P_H value of the cell sap of the different organs in late-flowering cosmos when exposed to the natural length of day and to a 10-hour day during the summer months (seed germinated May 24).*

Date of observation and part of plant used.	Plants exposed to natural length of day from date of germination.		Plants exposed to natural length of day from date of germination to June 28 and thereafter exposed to a 10-hour day.	
	Hour of sampling.	P_H value.	Hour of sampling.	P_H value.
July 12:				
Apex	9.15 a. m.	5.23	9.15 a. m.	[a] 5.56
Top leaves	1.35 p. m.	5.22	11.30 a. m.	5.48
Lower leaves	do	5.59	do	5.81
July 15 (and 17):[b]				
Apex	9.05 a. m.	5.18	9.15 a. m.	[a] 5.84
Top of stem	do	5.30	do	5.47
Middle of stem	11.15 a. m.	5.43	11.25 a. m.	5.47
Base of stem	do	5.46	do	5.43
Roots (fibrous)	1.50 p. m.	5.46	2 p. m.	5.46
July 20:				
Top leaves	2 p. m.	4.98	11.30 a. m.	5.15
Lower leaves	do	5.65	do	5.79
July 21:				
Apex	11.20 a. m.	4.98	9.15 a. m.	[a] 5.63
Top of stem	do	5.14	do	5.49

[a] Flower buds only.
[b] Observations of plants under the 10-hour day conditions were made on July 17.

To obtain further information on the relative acidity of the different organs of the plant as affected by the length of day, observations were made on plantings of cosmos which germinated May 24. One series was

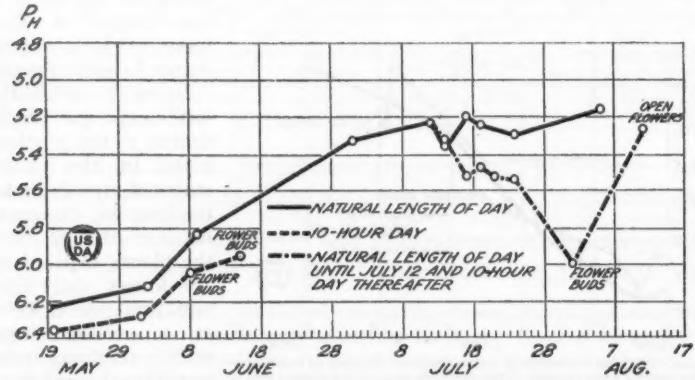

FIG. 5.—Showing the effect of abrupt change in light exposure from the long days of summer to a 10-hour day, on the active acidity of the sap in the apex of late-flowering cosmos (Table I). There is a prompt drop in active acidity to a level which is far below that characteristic of the advanced vegetative stage and the same as that exhibited in plants exposed to a 10-hour day from the outset. Here, again (compare fig. 4), the active acidity of the open blossom is practically identical with that of the apical bud in the advanced vegetative stage which results from a long-day exposure.

exposed to the natural length of day throughout the test and a second series was transferred from the full day length to a 10-hour day on June 28. Flower buds were found on the latter series on July 8. The data obtained are given in Table III. For the sample of top leaves the three

uppermost were used; for lower leaves the oldest available were taken, some of which had begun to yellow. The upper and lower leaves show much the same acidity relations as the corresponding portions of the axis and in all cases the acidity is lowest under the 10-hour day. The acidity of the fibrous roots is about the same as that of the base of the stem.

During the short days of winter electric light of intensity as low as 5 foot-candles used to prolong the day length is effective in preventing flowering in cosmos, and the observations recorded in Table IV (A) indicate that the acidity relations characteristic of the more advanced vegetative condition are thereby maintained. After flowering has been induced in cosmos by exposure to short-day conditions a return to the vegetative condition, involving phemomena of rejuvenescence, is eventually effected by exposure of the plant to long-day conditions. Cosmos which was planted in the greenhouse March 11 formed flower buds in response to the short seasonal length of day. The first blossom opened May 8. By May 21 it was evident that as a result of the increasing length of day the plants were passing definitely into the vegetative condition. Observations on these plants are recorded in Table IV (B). Seedlings of cosmos which germinated May 15 under a 10-hour day were showing flower buds by June 3. On June 13 these plants were placed permanently out of doors. By June 28 evidence could be seen of the change to the vegetative condition, though flowering had not entirely ceased. Transition stages in this type of rejuvenescence are shown in Plate 1, B. Observations on these plants are given in Table IV (B). It is obvious that change from short-day to long-day conditions after flowering has been initiated results in establishing the relatively high acidity of the apical structures which is characteristic of the advanced vegetative condition.

EXPERIMENTS WITH BILOXI SOY BEANS

The type of growth in soy beans differs markedly from that in cosmos in that stem elongation is decidedly reduced, there is much branching, and development of foliage in proportion to stem is greater. Late maturing varieties of soy beans, however, show much the same responses to length of day as does late cosmos. For study of acidity relations the young, topmost leaves averaging 1 to 1½ inches in length were commonly used. As the flowering condition is approached the formation of new leaves is checked, so that in such cases, while the leaves taken for study were of the usual size, they were probably somewhat older than those gathered during the more active vegetative period. In a series of observations on plants growing in the field during the months of June and July, which will not be reported here in detail, it was found that the acidity of the sap from the young, topmost leaves remained close to the P_H value 6.50 from the earliest stages of growth up to the approach of the flowering condition, while thereafter the acidity increased up to a maximum of P_H 5.97 after seed pods had appeared. Larger, more mature leaves showed a somewhat higher acidity. The acidity gradient of the stem and root during the vegetative period was found to be quite similar to that in cosmos. Typical P_H values found for top and base of stem and fibrous roots are 5.97, 6.17, and 6.46, respectively. As flowering buds came into evidence there was a temporary rise in P_H value in the top of stem to about 6.25, followed by a return toward previous values as the flowers unfolded.

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TABLE IV.—*Observations on late-flowering cosmos, showing: (A) the effectiveness of low-intensity electric light as a supplement to daylight in establishing the high hydrogen-ion concentration of the cell sap which is associated with the advanced vegetative condition, and (B) the rise in hydrogen-ion concentration of the upper parts of the plant in the return from the flowering to the vegetative condition (rejuvenescence) which results from exposure to increased length of day*

A			B					
Planted Nov. 1 (and Jan. 5) in the greenhouse and exposed to natural daylight supplemented with electric light to furnish 18 hours of illumination daily.			Planted Mar. 11 in greenhouse and exposed to natural increase in length of day.			Planted May 10 in boxes and exposed to a 10-hour day till June 13, and thereafter exposed to natural length of day.		
Date and hour of sampling.	Part of plant used.	P_H value.	Date and hour of sampling.	Part of plant used.	P_H value.	Date and hour of sampling.	Part of plant used.	P_H value.
Apr. 15, 9.20 a. m.	Topmost leaves	5.17	May 7, 2.35 p. m.	Leaves near top	6.23	June 10, 12.20 p. m.	Flower buds	6.10
Apr. 20, 11.40 a. m.	do	5.06	May 21, 2.50 p. m.	do	5.36	June 21, 12.50 p. m.	do	5.93
May 6, 1.35 p. m.	do	[a]5.21				June 28, 12 noon	Open blossoms	5.25
May 7, 12.25 p. m.	Apex	5.30				Aug. 11, 10.45 a. m.	Apex (vegetative)	5.24
May 22, 1.05 p. m.	Topmost leaves	5.17						

[a] Planted Jan. 5.

To study further the effect of the light period on acidity, plantings were made in six metal buckets of 12-quart capacity on June 30, and the seedlings were above ground on July 3. One series of three buckets remained out of doors throughout the test, while a second series of three buckets was permanently transferred from the full length of day to a 10-hour day on August 5. The latter series began flowering August 29. The former series began flowering September 15, although flower buds could be seen a week earlier. The results of the observations are shown in Table V and figure 10. These studies were only begun shortly before the plants passed into the flowering condition as a result of the natural decrease in length of day, for it is known that a minimum period of at least 3 weeks is required for the unfolding of the blossom after the plant comes under the influence of a favorable day length. It will be seen that under the natural length of day there is a decided decrease in acidity for about one week ending August 19, immediately followed by a sharp increase which continues progressively till after flowering has begun. Abrupt shortening of the daily light period to 10 hours had somewhat reduced the hydrogen-ion concentration at the end of 48 hours, but the full effect of the change did not come about till the end of 5 or 6 days. Thereafter there was a sharp progressive increase in acidity just as under the natural decrease in length of day. The effect of the 10-hour day, however, was to accentuate and to hasten these changes in acidity. Biloxi soy beans thus closely resemble cosmos in acidity relations except that the relatively reduced stem elongation characteristic of the soy beans in the vegetative condition is associated with a decidedly lower maximum acidity of the apical structures than is attained in cosmos, in which marked elongation of the axis takes place.

TABLE V.—*Showing the P_H value of the cell sap of the young, topmost leaves of Biloxi soy beans grown in buckets when exposed to the natural change in length of day in late summer and fall and when abruptly transferred from these conditions of illumination to a 10-hour day.*

Time at which sample was taken.		Plants exposed to natural length of day throughout the test.	Plants permanently transferred from the natural length of day to a 10-hour day on Aug. 5.
Date.	Hour.	P_H value.	P_H value.
Aug. 7	9.30 a. m.	6.28	6.44
Aug. 9	9.20 a. m.	6.31	6.52
Aug. 11	9.30 a. m.	6.41	6.95
Aug. 13	9.10 a. m.	6.56	7.00
Aug. 16	9.20 a. m.	6.36	6.60
Aug. 19	9.40 a. m.	6.70	6.51
Aug. 24	11.15 a. m.	6.33	6.36
Aug. 31	10.05 a. m.	6.32	.....
Sept. 1	12.15 p. m.	.....	6.21
Sept. 7	11.25 a. m.	6.29	6.27
Sept. 16	9.45 a. m.	6.27	6.07
Sept. 21	11 a. m.	6.08	.....

The preceding experiment was repeated with two rows of Biloxi soy beans planted in the field which had germinated May 19 and 21, respectively. Beginning July 24, a portion of the plants were allowed to receive only 10 hours of light daily, this being accomplished by placing over the plants a ventilated, light-proof box which could be opened and closed as desired. In this case the exposure to the shortened day length was not begun until just as the plants were passing over into the flowering condition as a result of the natural decrease in length of day. Flower buds could first be seen on August 10 under the 10-hour day and on August 22 under the natural length of day. The transition from the vegetative to the reproductive stage occurred considerably earlier than in the preceding experiment because the plants had been seeded much earlier. The results of the observations, presented in Table VI, are even more sharply defined than those of the preceding test with respect to the initial decrease in acidity, and bring out clearly the same general relationships as to the course of changes in acidity. This test emphasizes the fact that under the conditions important changes in acidity as a result of abrupt shortening of the day length do not occur till after the lapse of 2 or 3 days.

TABLE VI.—*Showing the P_H value of the cell sap of the young, topmost leaves of Biloxi soy beans growing in the field when exposed to the natural change in length of day in summer, and when the daily light period was abruptly changed from the natural daylight period of summer to a 10-hour period of illumination*

Date of sample.	Plants exposed to natural change in length of day throughout the test.		Plants exposed to natural change in length of day till July 24 and thereafter exposed to a 10-hour day.	
	Hour of sampling.	P_H value.	Hour of sampling.	P_H value.
	a. m.		a. m.	
July 22	9. 50	6. 37	.....	.....
July 25	9. 00	6. 38	10. 20	6. 37
July 27	9. 15	6. 80	10. 25	6. 57
July 29	8. 50	6. 27	10. 30	6. 39
Aug. 1	9. 15	6. 11	10. 30	6. 28
Aug. 4	10. 35	6. 20	9. 05	6. 20
Aug. 7	9. 25	6. 07	10. 45	6. 01
Aug. 10	10. 30	6. 20	9. 10	6. 03
Aug. 14	9. 35	6. 18	11. 00	6. 16
Aug. 17	10. 15	6. 04	9. 00	6. 08
Aug. 22	10. 00	6. 24	.....	.....
Aug. 25	9. 45	6. 07	.....	.....
Aug. 30	9. 50	6. 10	.....	.....
Sept. 8	9. 45	6. 06	.....	.....

Studies were made on acidity relations in the developing seed pods of Peking soy beans, which had germinated June 27, as representing the final features of reproductive activity. In the earlier stages of development the seed pods in their entirety were necessarily taken, but as soon as the seeds had attained sufficient size these were separated from the hulls. The samples were collected at 1.30 to 2.30 p. m. For analysis the seeds in the more mature stages were ground with distilled water. The results are shown in Table VII. The hulls, constituting the vegetative portion of the fruit, increase in acidity as they develop, while the

seeds show a much lower and decreasing acidity till about the time maximum size is attained. During the later stages of ripening the seeds appear to increase somewhat in acidity.

TABLE VII.—*Showing the P_H value of cell sap of seed pods and seeds in the Peking variety of soy beans at different stages of development*

Date of sample.	Material.	P_H value.
Aug. 31	Entire seed pods, less than ½ inch long	6.29
Sept. 1	Entire seed pods, about 1 inch long	6.09
Do	Entire seed pods, about 1¼ inches long	6.10
Sept. 9	Entire seed pods, about 1¾ inches long	6.24
Sept. 19	Hulls only, 1¼ to 1¾ inches long	5.94
	Seeds only	6.63
Sept. 28	Hulls only, 1½ to 1¾ inches long	5.91
	Seeds only, just beginning to turn red	6.83
Oct. 5	Seeds only, full size, seed coats red in color	6.63
Oct. 7	Seeds only, black, nearly dry	6.33
Oct. 28	Seeds only, dry and mature	6.34

EXPERIMENTS WITH MARYLAND MAMMOTH TOBACCO

This variety of tobacco behaves like the late varieties of cosmos and soy beans in response to length of day, and observations made on acidity relations gave results similar to those already presented for cosmos and soy beans. Typical P_H values obtained for the terminal bud and the topmost and basal portions of the stem, respectively, are 5.25, 5.53, and 5.30 during the vegetative stage of the plant, and 5.46, 5.51, and 5.29 just after the flower bud became visible. The acidity gradients of the stem under the two conditions are similar to those obtained for cosmos, though not quite so pronounced.

EXPERIMENTS WITH TITHONIA ROTUNDIFOLIA (MILL) BLAKE

This tropical weed attains giant proportions without flowering when grown during the summer months in the latitude of Washington, but flowering is readily induced by shortening the length of day. Studies were made on the acidity of the cell sap as affected by the daily light period. Seed were planted May 16 and the seedlings transferred to boxes on June 20. One series was exposed to the natural length of day throughout the test, a second series was permanently transferred from the natural length of day to a 10-hour day on July 12, and a third series was similarly transferred to a 10-hour day on August 17. Determinations of hydrogen-ion concentration in the topmost and basal portions of the stem were made, using for the purpose sections 1½ to 2 inches in length. Under the natural length of day no flower buds could be seen as late as October 9, while they were first visible July 26 in the plants transferred to the 10-hour day on July 12 and were first seen on September 6 in the plants similarly transferred on August 17. The results of the acidity measurements are summarized in Table VIII. Under the long days of summer the acidity of the stem increased steadily up to August 22 but thereafter the decreasing length of day caused progressive decrease in acidity (fig. 6). The first transfer of plants to the short day was made before the acidity had increased to that associated with the advanced vegetative condition, and

under these circumstances a slight increase in acidity as compared with the plants remaining under the full length of day was found on the sixth day after the transfer. A week later, however, the plants exposed to the longer daylight period had become the more acid. Transfer of the plants to the 10-hour day after the advanced vegetative stage had been reached had the effect of bringing about a transitory decrease in acidity about the fifth day after the transfer had been made. This was soon followed by a moderate increase in acidity prior to the appearance of the flower bud.

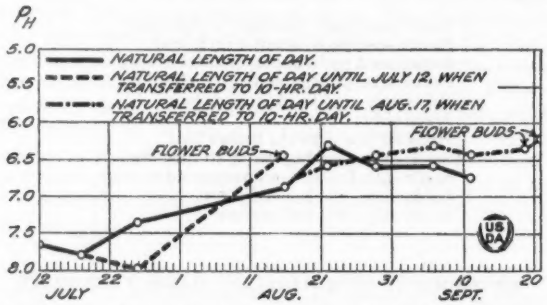

FIG. 6.—Showing the active-acidity relations in the uppermost portion of the stem in *Tithonia rotundifolia* when exposed to the long days of summer and to a 10-hour day, respectively (Table VIII). These relationships are very similar to those found in cosmos. Abrupt decrease in the light period causes a decrease in active acidity, followed by rise during the development of the flower bud.

TABLE VIII.—*Showing the P_H value of cell sap in topmost and basal portions of the stem in Tithonia rotundifolia as affected by the duration of the daily illumination period*

Date of sample.	Plants exposed to natural length of day.				Plants transferred from the natural length of day to a 10-hour day.[a]			
	Hour of sampling.		P_H value.		Hour of sampling.		P_H value.	
	Top of stem.	Base of stem.	Top of stem.	Base of stem.	Top of stem.	Base of stem.	Top of stem.	Base of stem.
July 12	8.45 a. m.	10.20 a. m.	7.64	6.46				
July 18	11.55 a. m.	2.25 p. m.	7.78	6.78	1.10 p. m.	3.25 p. m.	7.79	6.26
July 26	9.05 a. m.	12.20 p. m.	7.34	5.96	10.50 a. m.	1.35 p. m.	7.96	6.04
Aug. 16	9.10 a. m.	1.35 p. m.	6.87	5.63	10.25 a. m.	3 p. m.	[b] 6.44	5.74
Do					12.20 p. m.		[c] 6.34	
Aug. 22	12.10 p. m.		6.30		1.25 p. m.		6.57	
Aug. 29	1.20 p. m.		6.60		12.20 p. m.		6.46	
Sept. 6	12.25 p. m.		6.59		1.30 p. m.		6.32	
Sept. 11	2.25 p. m.		6.76		1.20 p. m.		6.43	
Sept. 19					3 p. m.		[b] 6.36	
Sept. 21					1 p. m.		[b] 6.23	

[a] The data for the period July 18 to Aug. 16, inclusive, relate to plants permanently transferred from the natural length of day to a 10-hour day on July 12; subsequent data relate to plants similarly transferred on Aug. 17.
[b] Flower buds about one-half inch in diameter.
[c] Upper portion of flower bud stem.

EFFECT OF THE LIGHT PERIOD ON ACIDITY RELATIONS IN LONG-DAY PLANTS

In plants of this group exposure to relatively long days results in stem elongation, which is invariably coupled with flowering; that is, the condition of indeterminate elongation of the sterile vegetative stem is wanting. Exposure to relatively short days tends to inhibit stem

elongation and flowering, thus resulting in the leaf-rosette type of development, either with or without tuberization. Radish (*Raphanus sativus* L.) may be taken as an example of this type in which a short daily illumination period results in marked tuberization without stem elongation, while *Rudbeckia bicolor* L. may be taken to represent the type in which tuberization does not result under these conditions.

EXPERIMENTS WITH SUMMER RADISH

Radish of the horticultural variety Scarlet Globe was planted in wooden boxes and galvanized-iron cans in the greenhouse on September 29 and had germinated on October 3. One lot of plants was exposed to the natural length of day while the other lot received, in addition, illumination from 50-watt electric bulbs without reflectors, placed about 18 inches above the plants. The electric lights were turned on at sunset and turned off at midnight, the plants thus receiving about 18 hours of illumination daily. By December 6 development of the primary axis had begun in 46 individuals, or 82 per cent, of the plants exposed to the 18-hour day, and under the natural length of day 9 plants, or 17 per cent, of the total were developing a stem. Of the two lots the plants under the longer illumination period were much the taller. While the natural length of day of winter at Washington is not short enough to permanently inhibit stem elongation in this radish, the process is much delayed and restricted.

Results of observations on the hydrogen-ion concentration of the sap of the plants under the two conditions are shown in Table IX. For the leaf material the entire midribs of the larger leaves were used after removal of the lamina. In sampling the tuber or thickened root approximately the upper third was taken for the first sample and for the second sample a central horizontal section constituting approximately a third of the total was used, the lower third with tap root being rejected. In sampling the primary stem, in the case of the plants exposed to the longer illumination period, the topmost section of 1 inch in length and a section one-half inch in length immediately above the thickened root were used. For the samples taken on December 15 plants were used which had developed stems 9 inches high, while the plants used four days later had stems only 6 inches high. The plants used January 4 had developed stems 32 inches high which were showing flower buds. It may be added at this point that unopened flower buds collected January 5 from somewhat further advanced plants showing open blossoms gave a P_H reading of 5.51. In the case of plants exposed to the natural length of day only those were used which showed no evidence of developing flowering stems. In this instance the acidity values corresponding to those of the top of the stem in the plants under the lengthened light exposure relate to the tissues in the region of the growing point immediately above the thickened root after removal of the leaves of the rosette. The data in Table IX show that under the relatively short daily illumination period, which is unfavorable for stem elongation, the acidity of the plant as a whole is relatively low and is lower in the region of the growing point than elsewhere. Under the longer light period the acidity of the plant is higher, especially in the upper part of the stem, which increases in acidity as growth proceeds. It may be added here that while the average weight of tops of the plants under the longer daily illumination was much greater, the average weight of roots was decidedly less than the corresponding

TABLE IX.—*Showing the P_H value of the cell sap of summer radish when exposed to the natural length of day of winter and to the natural daylight period supplemented with electric light to give 18 hours total illumination daily*

Part of plant used.	Plants exposed to natural length of day of winter.						Plants exposed to natural daylight supplemented with electric light so as to furnish 18 hours of illumination daily.					
	Dec. 14.		Dec. 17.		Jan. 6.		Dec. 15.		Dec. 19.		Jan. 4.	
	Hour of sampling.	P_H value.	Hour of sampling.	P_H value.	Hour of sampling.	P_H value.	Hour of sampling.	P_H value.	Hour of sampling.	P_H value.	Hour of sampling.	P_H value.
Top of primary stem[a]	11.20 a. m.	6.38	11.15 a. m.	6.55	10.45 a. m.	6.49	11.20 a. m.	5.94	11 a. m.	6.20	11.30 a. m.	5.27
Base of primary stem							do	6.14	do	6.38	1.10 p. m.	6.33
Stem of leaf	9.55 a. m.	6.08	10.05 a. m.	6.06	9.45 a. m.	5.96	10 a. m.	5.92	9.40 a. m.	5.87	10.10 a. m.	5.80
Upper portion of tuber	1.15 p. m.	6.44	1 p. m.	6.46	12.40 p. m.	6.29	2.05 p. m.	6.10	1.45 p. m.	6.29	2 p. m.	6.06
Central section of tuber	do	6.01	do	5.85	do	5.72	do	5.97	do	5.96	do	6.03

[a] In case of plants exposed to natural length of day samples consisted of the tissues in the region of the growing point situated immediately above the thickened root, after removal of the leaves, there having been no considerable elongation of the axis.

parts of the plants exposed to the shorter illumination period. The above results of the studies on acidity relations were confirmed by observations on plants exposed to the full length of day of late spring and summer in comparison with plants exposed to a 10-hour day, but it seems unnecessary to present the data in detail. In these latter experiments the plants germinated April 13. As late as June 7 the very young leaves of the rosettes in the plants exposed to a 10-hour day showed a P_H reading of 6.06 while the tops of the stems of the plants exposed to the natural length of day (nearly 15 hours), which were showing young flower buds, gave a reading of 5.57. By June 26 stem elongation was beginning in the plants under the 10-hour day and the tops of the stems showed a P_H reading of 5.67.

EXPERIMENTS WITH RUDBECKIA BICOLOR.

Seeds of this garden ornamental were planted in the greenhouse December 19 and on January 27 the seedlings were transplanted in flats. On February 6 one lot of the small seedlings was transferred from the natural length of day to a 7-hour day. A second lot was exposed to an 18-hour daily illumination, a 100-watt electric bulb being placed 2 feet above the plants and the current turned on at sunset and turned off at midnight each day. On March 22 a portion of the plants which had been exposed to the 7-hour day were permanently transferred to the 18-hour day. Under the 7-hour day stem elongation was indefinitely inhibited, but under the 18-hour day development of the axis had begun within 2 weeks. Growth characteristics under the two light conditions are shown in plate 2,A. Of the plants under the 7-hour day, only the leaves, of course, were available for acidity studies. In sampling, all sound leaves excepting the smallest individual of the rosette were used in both the long-day and the short-day series of plants. The leaves of the plants exposed to the 18-hour day were decidedly longer than those of the plants under the 7-hour day. In sampling the stems of the plants under the 18-hour light period the entire stem was used when the length was less than 4 inches. In all other cases 2-inch sections were used. In sampling the flower bud the involucre was rejected. The results of the observations are summarized in part in Table X. Additional observations which need not be reported in detail have confirmed the data here given. It may be added that samples of open blossoms and upper and lower portions of stems collected May 10 from plants which had been exposed to the 18-hour light period from February 5 gave P_H readings of 5.55, 5.41, and 5.68, respectively.

The acidity relations of the leaf in Rudbeckia differ somewhat from those in radish under the two conditions of illumination. In radish the higher acidity occurred under the longer light period, while the reverse was true in Rudbeckia. Again, under the influence of the longer light period maximum acidity of the stem in radish is found in the upper portion even before the appearance of the flower buds. In Rudbeckia the topmost portion of the stem is consistently less acid than the basal portion up to and including the period of flower formation (fig. 7). This relationship is not reversed till the blossom has unfolded. In both radish and Rudbeckia, however, the acidity of the upper portion of the stem increases progressively prior to the appearance of flower buds, although in both the increase is more pronounced in subsequent development.

TABLE X.—*Showing the P_H value of the cell sap of Rudbeckia bicolor exposed to a 7-hour day and an 18-hour day*

Date of sample.	Plants exposed to a 7-hour day.		Plants exposed to an 18-hour day.[a]						
	Hour of sampling leaf.	P_H value of leaf.	Height of stem.	Hour of sampling.			P_H values.		
				Leaf.	Top of stem.	Base of stem.	Leaf.	Top of stem.	Base of stem.
			Inches.						
Feb. 24	12.40 p. m.	6.62	1¾	2 p. m.	3 p. m.	.....	6.93	[b] 7.69	.....
Do	.....	.....	2¾	.....	3.40 p. m.	.....	.....	[b] 7.32	.....
Feb. 26	10.05 a. m.	6.61	1¾	11.10 a. m.	1.05 p. m.	.....	6.94	[b] 7.43	7.30
Do	.....	.....	4	.....	2 p. m.	3.10 p. m.	.....	7.89	.....
Mar. 3	.....	.....	6	.....	10.30 a. m.	11.50 a. m.	.....	7.72	7.03
Mar. 10	.....	.....	10	.....	11.40	12.55 p. m.	.....	7.66	7.04
Mar. 19	.....	.....	17	12.40 p. m.	1.40 p. m.	3.40 p. m.	[c] 6.48	7.24	6.55
Mar. 21	10.10 a. m.	6.58	.....	.....	.....	.....	.....	.....	.....
Apr. 12	.....	.....	7	.....	9.55 a. m.	11.10 a. m.	.....	7.17	5.91
Apr. 23	.....	.....	18½	11.25 a. m.	12.55 p. m.	1.55 p. m.	[c] 6.37	6.63	5.89
Apr. 27	.....	.....	20	9.50 a. m.	10.55 a. m.	12.45 p. m.	[c] 6.01	6.27	5.79

[a] The data for the period Feb. 24 to Mar. 19, inclusive, relate to plants exposed to 18 hours of light daily from the beginning of the experiment; the data for the period Apr. 12 to 27, inclusive, relate to plants exposed to the 7-hour day till Mar. 22 and thereafter exposed to the 18-hour day.

[b] Entire stem.

[c] Flower buds.

It is of considerable interest to compare the acidity relations in Rudbeckia, as representative of the group of long-day plants which do not ordinarily become tuberized, with the acidity of the short-day plants. The course of development of long-day plants when exposed to relatively long days is more or less similar to that of short-day plants when exposed to relatively short days. By comparing the acidity in the stem of Rudbeckia under the 18-hour day (Table X) with that of cosmos (Table II) and Tithonia (Table VIII) under a 10-hour day it will be apparent that in general the acidity relations are very similar. There is in all cases an increase in acidity of the stem till the flower bud appears. The flower buds themselves increase in acidity as growth proceeds, maximum acidity being found in the unfolded blossom. In distribution of acidity the upper portion of the stem is in all cases less acid than the lower portion, in contrast with conditions in the short-day plants when exposed to long days. Radish, as representative of the group of long-day plants which commonly become tuberized under short days, seems to differ somewhat from short-day plants (when exposed to short days) and the Rudbeckia group of long-day plants in distribution of acidity in the stem, particularly in the earlier stages of stem development.

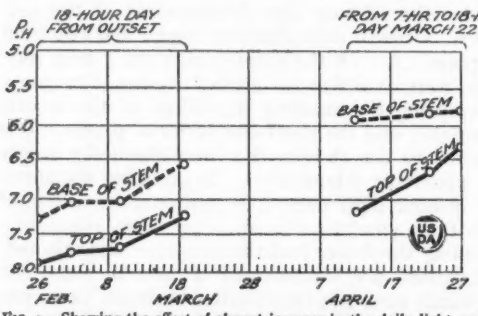

FIG. 7.—Showing the effect of abrupt increase in the daily light period on the active acidity of the cell sap in the stem of Rudbeckia bicolor, which is a typical long-day plant (Table X). Increase in the light period from 7 hours to 18 hours initiates elongation of the axis which is followed by flowering. In this group of plants the upper portion of the stem is lower in active acidity than is the base, a condition which is found in short-day plants only during the early stages of development and in the initial stages of reproductive activity. Thus, in the typical long-day plants the level of active acidity characteristic of the flowering stage is attained by an increase rather than a decrease in acidity of the stem apex.

ACIDITY RELATIONS IN PLANTS OCCUPYING AN INTERMEDIATE POSITION BETWEEN THE MORE TYPICAL SHORT-DAY AND LONG-DAY PLANTS

There is a group of plants which may be regarded as standing between typical short-day and long-day plants in their response to differences in length of day. In these plants the height attained increases with increase in the daily illumination period, but the condition of indeterminate elongation of the vegetative stem is not readily attained under relatively long days, nor is stem elongation readily and completely inhibited by exposure to short days. *Helianthus annuus* L. is a representative of this group. The range in length of day during the open growing season at Washington greatly affects the height attained by this sunflower but does not materially influence the time required for flowering. On the other hand, a more extreme change in the light period causes appreciable change in time of flowering and, of course, further accentuates differences in height of the stem. The effect of the light period on the height of the plant is shown in plate 2, B.

A series of plantings of sunflower were made at intervals of two weeks through the summer months. Observations were made on heights attained by the plants, the dates of flowering and the hydrogen-ion concentration of the cell sap at different stages of development. The data on height attained and time of flowering are shown in Table XI. The maximum height was reached by the first two plantings, but unfortunately exact data were not secured on these plantings. The plantings made after August 25 failed to flower because of onset of cold weather.

TABLE XI.—*Number of days from germination to flowering and final height attained by Helianthus annuus when planted at two-week intervals during the summer months*

Date planted.	Date of germination.	Date of first open blossom.	Number of days from germination to flowering.	Average height of plants at time of flowering.
				Inches.
June 15	June 29	Aug. 19	51	.....
June 29	July 5	...do	45	.....
July 13	July 19	Sept. 6	49	66
July 27	Aug. 2	Sept. 19	48	60
Aug. 11	Aug. 18	Oct. 2	45	50
Aug. 25	Sept. 2	Oct. 24	52	42

Results of observations on acidity relations in the different plantings at given stages of development and of individual plantings at various stages of development are shown in Table XII. For the leaf samples the three topmost leaves, exclusive of the embryonic leaves of the bud, were used. A 2-inch section of the stem immediately below the apical bud and a similar section immediately above the ground level were used. The data are consistent and establish several points of interest. Under the seasonal range in length of day at Washington the response of this sunflower closely coincides with that of cosmos and Tithonia when these are exposed to short days. Exactly the same relationship holds with respect to acidity of the cell sap. In the first section of Table XII data are given for seven different plantings of sunflower at 2-week intervals, and it is apparent that the seasonal difference in length of day does not materially affect the acidity of the plant during the first 3 weeks of growth.

Throughout this period the acidity of the tissues in the region of the apex is decidedly less than that of the lower portion of the stem (fig. 8). In all plantings flower buds could be seen about 30 days after germination. The data in the second section of the table show the changes in acidity which are associated with the appearance and development of the flower bud. In earlier stages of development acidity relations in the young, topmost leaves, the upper portion of the stem, and the growing point are much the same. The appearance of the flower bud, however, is marked by considerable increase in acidity in the upper portions of the plant and the flower bud increases progressively in acidity as growth proceeds (fig. 8). Additional observations on other field plantings show further increase in acidity till a P_H value as low as 5.22 is reached in the fully expanded blossom. On the other hand, as the seeds develop these show decreasing acidity till in large well-filled seeds the P_H value rises to approximately 7.05.

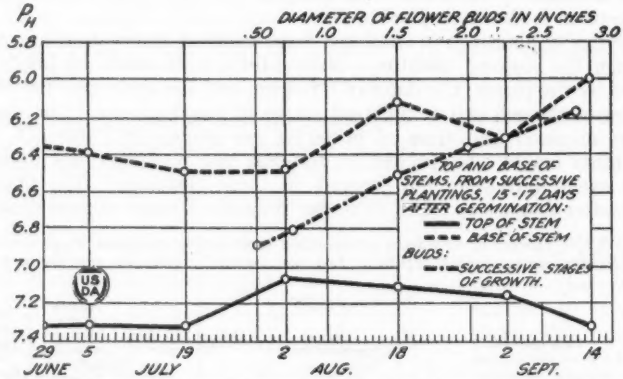

FIG. 8.—Showing the effect of the natural change in length of day of summer and early fall on the active acidity of the top and base of the axis in sunflower and the change in acidity during the development of the flower bud (Table XII). Sunflower resembles the typical long-day plants in that within the seasonal range in length of day (at Washington) the active acidity at the top of the stem is less than that of the base. The flower bud increases sharply and regularly as growth proceeds.

TABLE XII.—*Showing the P_H value of the cell sap of Helianthus annuus planted at intervals of two weeks during the summer and at different stages of development*

Date of germination.	Date of sample.	Number of days after germination when sampled.	Hour of sampling.	P_H value of sap.					Diameter of flower bud.
				Topmost leaves.	Top of stem.	Base of stem.	Apical bud.	Flower bud.	
									inches.
June 29	July 12	13	11.30 a. m.	7.16	7.33	6.37			
July 5	July 22	17	11.35 a. m.	7.25	7.32	6.39			
July 19	Aug. 3	15	12.30 p. m.	7.74	7.33	6.50			
Aug. 2	Aug. 19	17	1.30 p. m.	7.19	7.06	6.49			
Aug. 18	Sept. 2	15	1 p. m.	7.05	7.11	6.12			
Sept. 2	Sept. 17	15	12.35 p. m.	7.15	7.15	6.31			
Sept. 14	Sept. 29	15	9.50 a. m.	7.12	7.32	5.99			
Aug. 18	Sept. 2	15	1 p. m.	7.05	7.11	6.12			
Do	Sept. 9	22	9.50 a. m.				7.11		
Do	Sept. 13	26	2.50 p. m.				7.28		
Do	Sept. 15	28	9.20 a. m.-11.50 a. m.		7.17	6.15	7.11		
Do	Sept. 23	36	9.50 a. m.					6.89	1/2
Aug. 2	Sept. 9	38	9.50 a. m.					6.81	3/4
Do	Sept. 13	42	1.15 p. m.					6.52	1 1/2
Do	Sept. 14	43	9.50 a. m.		6.83			6.36	2
			1.30 p. m.			6.13			
Do	Sept. 30	59	2.30 p. m.					6.17	2 3/4

Observations on plantings which germinated in the greenhouse on January 23 and were exposed to the natural daylight period and to 18 hours of daily illumination, respectively, are recorded in Table XIII. The lengthened illumination period was maintained by use of 100-watt lamps from sunset till midnight, the lamps being placed about 1 foot above the plants. First flower buds were showing on the plants exposed to the natural light period on March 1, or 37 days after germination, and under the 18-hour period first flower buds were seen March 22, or 58 days after germination. For all observations prior to April 6, however, only plants which had not formed flower buds were used, except that one of the four individuals in the short-day series used on March 14 contained a very small bud. It is evident that 18 hours of light daily, as compared with the natural daylight period ranging from about 9½ to 12½ hours, materially delayed the time of flowering and increased the height of the stem. It is clear, also, that the longer light period maintained a higher level of acidity in the upper portions of the plant than did the shorter light period (fig. 9). There is, therefore, a tendency for the sunflower when exposed to an 18-hour light period to show the growth and acidity relations of typical short-day plants like cosmos and Tithonia when exposed to long days. The data in Tables XII and XIII, taken together, show that *Helianthus annuus* forms a connecting link between short-day and long-day plants, since it shows some of the characteristics of both types.

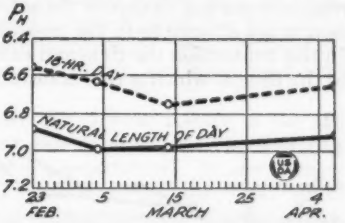

FIG. 9.—Showing the increase in level of active acidity of the apical structures in sunflower when the daily light period is increased to 18 hours. (Table XIII.) Under these conditions flowering is delayed, and in this respect as well as in the increased active acidity of the apex sunflower approaches the behavior of typical short-day plants such as cosmos. Thus, sunflower may be regarded as a connecting link between the more typical short-day plants on the one hand and the typical long-day plants on the other.

TABLE XIII.—*Showing the P_H value of cell sap of Helianthus annuus exposed to the natural length of day during the winter and early spring and to 18 hours of daily illumination.*

PLANTS EXPOSED TO THE NATURAL LENGTH OF DAY.

Date.	Height.	Hour of sampling.			P_H values.		
		Apical bud.	Top of stem.	Base of stem.	Apical bud.	Top of stem.	Base of stem.
	inches						
Feb. 23	11½	.....	10.50 a. m.	1.55 p. m.	.....	6.88	5.99
Mar. 4	17¼	10.50 a. m.	.....do.....	2.55 p. m.	7.04	6.99	6.09
Mar. 14	24½	10.55 a. m.	1.15 p. m.	3.20 p. m.	6.81	6.98	6.13
Apr. 6	56½	10.45 a. m.	1.30 p. m.	4 p. m.	[a]6.44	6.91	6.18

PLANTS EXPOSED TO 18 HOURS OF ILLUMINATION DAILY.

	inches						
Feb. 23	20	.....	10.10 a. m.	12.55 p. m.	.....	6.56	5.78
Mar. 4	31¼	9.50 a. m.	1.05 p. m.	2.55 p. m.	6.82	6.63	6.10
Mar. 14	45½	9.55 a. m.	12.15 p. m.	2.20 p. m.	6.73	6.76	6.16
Apr. 6	86¼	9.45 a. m.	12.35 p. m.	2.50 p. m.	[a]6.50	6.65	6.17

[a] Young flower buds, 1 to 1½ inches in diameter.

ACIDITY RELATIONS DURING THE PERIOD OF TRANSITION FROM THE VEGETATIVE TO THE FLOWERING CONDITION

The data which have been presented in the preceding paragraphs show that decided change in the hydrogen-ion concentration of the cell sap is closely associated with change in the course of development of the plants as induced by increasing or decreasing the duration of the daily illumination period. Thus, change from long to short days, which promptly initiates flowering in such plants as cosmos and Biloxi soy beans, also results in marked change in the active acidity of the plant sap. The question arises at once as to the possible significance of the change in acidity. In this connection the time and extent of the change in acidity in relation to the time at which actual transition from one type of activity to another takes place is of special interest. It is obvious that abrupt change from long to short days acts very quickly in initiating the formation of flower primordia in typical short-day plants, so that any causal factor must be operative within a few days after the change in light exposure occurs. It has already been pointed out that decided change in acidity usually becomes apparent about three to five days after the change in light conditions has been made. At this time there is a sudden and marked decrease in acidity. This decrease, however, is only temporary and is promptly followed by abrupt increase in acidity to approximately the original level. This is followed by a further, slow increase during the development of the reproductive organs.

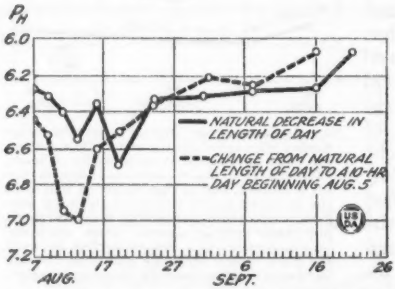

FIG. 10.—Showing the temporary, sharp decrease in active acidity of the cell sap in Biloxi soy beans which results from suitable decrease in the daily illumination period (Table V). This change in active acidity apparently marks the transition from the vegetative to the reproduction stage. The abrupt shortening of the daylight period produces a more decisive effect both in the temporary fall and in the subsequent rise in active acidity. The gradual, natural decrease in length of day seems to cause a less decisive wavering effect, probably indicating a rather delicate balance between the alternative vegetative and reproductive forms of activity.

These changes in acidity in Biloxi soy beans, as recorded in Table V (p. 137), are brought out more clearly in figure 10. It is believed that the sharp decline in acidity of the sap, occurring in this case 5 to 8 days after transfer to the 10-hour day, definitely marks the actual transition from the vegetative to the flowering stage. It will be observed that a similar but somewhat less pronounced decrease in acidity occurred a few days later in the plants exposed to the natural decrease in length of day. These plants began flowering September 15 and it is known that after suitable change in length of day has occurred a minimum period of about 25 days must elapse before the appearance of open blossoms. Hence, initiation of flower-bud formation must have been induced at about the time of the sharp decline in acidity. Since the natural decrease in length of day is gradual, it is to be expected that a critical point must occur at which there will be a condition of unstable equilibrium. A glance at figure 10 will disclose distinct evidence of such a wavering or "wobbling" action, the final transition from vegetative to flowering condition apparently occurring about August 19, just 27 days before the appearance of open blossoms.

In the preceding experiment the soy beans were not planted till July 1 and consequently under the natural length of day flowering began about 10 days later than the normal date of flowering for earlier plantings. In repeating the experiment, the results of which are shown in Table VI (p. 138), plantings were made May 15, and in this case flowering began September 4 under the natural length of day. The changes in acidity as shown in Table VI are much the same as in the previous experiment, under both the natural length of day and the 10-hour day. In this instance flowering apparently was initiated about July 27, but, because of the greater length of the day as compared with that prevailing at the transition period in the preceding experiment, 39 days were required for the appearance of open blossoms. This is in agreement with previous experience (see *8, p. 882*).

There is no satisfactory evidence that the drop in acidity of the sap which occurs during the transition from the vegetative to the flowering stage stands in a causal relation to this phenomenon. It seems more likely that the sudden decrease in acidity marks a temporary cessation of metabolic activity of the type concerned in stem elongation and of which the acidity itself is a product. It is to be remembered that light is not necessary for the decomposition of organic acids in the plant, while, on the other hand, exposure to light does result in the formation of these acids. The above results, if they have been correctly interpreted, are of interest, however, in indicating the triggerlike character of the transition from the vegetative to the flowering condition and furnishing a means of recognizing the very early stages in the series of transformations involved. It is quite possible, moreover, that these changes in acidity, as a link in the chain of events set up, in the first instance, by change in the light period, have important end results in influencing plant metabolism. The acidity relations in the plants under the 10-hour day in comparison with those under the natural length of day, as brought out in figure 10 and Tables V and VI, are in full agreement with the observed responses of the plants under the two conditions. Similar relationships are seen, also, in the data obtained with cosmos, Tithonia, and other species.

CARBOHYDRATE RELATIONS IN PHOTOPERIODISM

In view of the attention which plant physiologists have given in recent years to carbohydrate content of the plant as a factor in flowering and fruiting it seems desirable to include in the present paper the results of preliminary observations on the influence of length of day on the concentration of soluble carbohydrate in the tissues of the plant. Discussion of the literature on the general subject of carbohydrate content in relation to sexual reproduction is deferred till more complete data are available on effect of duration of the light period on the carbohydrate relations of the plant. In this connection mention should be made of recent work by Nightingale (*21*) dealing with the effect of the light period on the behavior and the chemical composition of certain plants. This author suggests that the duration of the light period is a factor in the utilization of carbohydrate for the conversion of nitrate nitrogen into protein form. In certain varieties of buckwheat, soy beans, radish, and salvia a 7-hour day limited the synthesis of nitrates to insoluble forms of nitrogen, even though an available supply of carbohydrate was present. In tomato plants already containing an abundance of carbohydrates shortening the light period caused marked decrease of carbohydrate

content coupled with decomposition of insoluble nitrogen. Under these conditions there was new growth, although there was no external supply of nitrates. As bearing on these relationships it may be pointed out that the relative capacity to effectively utilize carbohydrate for promotion of growth, the physiological translocation of plastic nutrients upward or downward in the plant, and the "reworking" of nitrogen and other plant-food elements present in limited quantities as a result of appropriate change in the light period have been discussed in a previous paper (*8, pp. 896, 901*) in so far as these phenomena are made evident by the observed responses of the plants.

Biloxi soy beans were planted June 29 in the field and samples of leaves were collected at intervals of two to three weeks for determination of soluble carbohydrates and nitrogen in the cell sap. The plants began flowering September 12. The leaf samples consisted of sets of three upper full-size leaves of the plant. The samples were collected at 10.30 a. m. The material was frozen and the sap expressed and filtered. To preserve the sap for sugar determinations, alcohol was added in quantity to form a 60 per cent solution after mixing, while for preserving the samples for nitrogen determination sulphuric acid was added to form a 4.5 per cent solution after mixing. Reducing sugars were determined, before and after inversion with hydrochloric acid, according to the methods for foods and feeding stuffs of the Association of Official Agricultural Chemists. The nitrogen determinations were made according to the official Kjeldahl method modified to include nitrogen in nitrates. The results are shown in Table XIV. So far as may be judged by these data there is a slight increase in reducing sugar in the leaf at about the time flower buds are laid down, followed by a decrease during the period of unfolding the first blossoms. Three weeks after the flowering stage is reached, however, when seed development is well under way, there is a marked increase in reducing sugar and a small increase in soluble nitrogen. There appears to be little if any polysaccharid present in the sap at any stage. The data are not sufficient to justify final conclusion, but as far as they go seem to indicate that the most marked accumulation of soluble forms of carbohydrate and nitrogen in the leaf takes place after flowering has been initiated and during the active development of the fruit.

TABLE XIV.—*Sugars and nitrogen contained in 10 cc. portions of sap of Biloxi soy beans taken at intervals during the growing season.*

Date of sample.	Reducing sugars.			Nitrogen.
	Before inversion. (a)	After inversion. (b)	Difference. (b–a)	
	Gm.	*Gm.*	*Gm.*	*Gm.*
July 26	0.0934	0.0954	0.002	0.0219
Aug. 16	.1030	.1060	.003	.0247
Sept. 7	.0750	.0786	.0036	.0226
Sept. 21	.0764	.0766	.....	.0236
Oct. 6	.1692	.1764	.0072	.0290

Late-flowering *Cosmos bipinnatus* was planted in boxes out of doors on May 10. On August 5, when the plants were about 60 inches high, one lot was placed under a 10-hour day. Under the 10-hour day flower

buds were first seen on August 17 and under the natural length of day they were observed on September 21. Samples for analysis, consisting of upper sections of the stems 18 inches in length, were collected at intervals, the attached leaves being removed and rejected. The samples were taken at 1 to 2 p. m. After taking the green weights of the stems, these were cut into 2-inch pieces and plunged into boiling alcohol to which a small quantity of calcium carbonate had been added. Boiling was continued for 15 minutes. Extraction of sugars from the stem material was completed with 50 per cent alcohol. Reducing sugar was determined before and after inversion. The water content of the samples was obtained by drying the extracts and residues at 100° C. and deducting the weights from the green weights of the samples. The results of the analyses are given in Table XV. There is in these data some indication of change in water content of the tissues as a result of change in the light period. Apparently a slight decrease in water content had taken place four days after the transfer to a 10-hour day had been made, while 12 days later, after flower buds had appeared, the water content had risen to 2 per cent above that of the plants exposed to the natural length of day. In this connection it is to be noted that this increase in water content occurs at a time when exceedingly rapid elongation of the flower stems takes place.

TABLE XV.—*Water and sugars contained in the upper portion of the stem of late-flowering cosmos as affected by transfer August 5 from the natural length of day of summer to a 10-hour day.*

Date.	Water content.		Total reducing sugar after inversion.		Reducing sugar before inversion.		Sucrose.	
	Natural length of day.	10-hour day.	Natural length of day.	10-hour day.	Natural length of day.	10-hour day.	Natural length of day.	10-hour day.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent*	*Per cent.*
August 7.....	84.95	85.18	18.41	21.02	8.15	10.40	9.75	10.09
August 9.....	85.00	84.64	14.02	20.80	6.37	6.85	7.27	13.25
August 21.....	84.89	86.74	16.25	20.75	7.79	10.79	8.04	9.46

It is plain that shortening the light period had a prompt and decided effect on the content of soluble carbohydrate. Two days after the change had been made there had been an increase amounting to 14 per cent of total sugars originally present. Thereafter, total sugars remained practically constant under the 10-hour day, although there was considerable fluctuation under the longer length of day. It will be observed that transfer to the short-day conditions affected the relative percentages of monosaccharid and polysaccharid. The increase of carbohydrate within the first 48 hours consisted almost entirely of reducing sugar, while 2 days later, with the total sugar content remaining constant, there was a marked increase in polysaccharid at the expense of the reducing sugar originally present. This change in proportion of monosaccharid to polysaccharid seems to be temporary, and subsequently the tendency is toward a return to a more nearly equal distribution between the two classes of sugars. Further studies are under way to determine whether these relationships are characteristic features of the effect of the light period on internal conditions of the plant in association with

change from the vegetative to the flowering condition induced by the light factor. There is no apparent reason for supposing that these changes in carbohydrate content are directly connected with photosynthesis, since the increase in soluble carbohydrate is caused by decrease in the duration of the illumination period.

Scarlet Globe radish was planted in the greenhouse September 29, and one lot of plants was allowed to grow under the natural length of day while a second lot was exposed to an 18-hour day, using electric light to prolong the daylight period. On January 10 the two lots of plants were sampled for determination of reducing sugar in the sap. Under the short day the plants sampled had not developed flowering stems but under the long day the plants had developed stems 20-24 inches high and these were showing flower buds at the tips. In sampling the thickened root only a central horizontal section was used. In the leaf material only the stem was used, the lamina being discarded. The stems were divided into upper and lower portions, the point of division being somewhat below the center. Leaf samples were taken at 10.30 a. m. and the other material at 1 p. m. The saps were prepared by grinding and pressing the material without freezing and then filtering the expressed juice. The results of the analyses are shown in Table XVI. In brief, it seems that under the longer light period the content of reducing sugar is considerably greater than under the relatively short light period and the concentration in the upper portion of the stem is much greater than in the lower. Nightingale (*21*) also found rapid upward translocation of carbohydrate in radish exposed to a long day. Apparently the increase in reducing sugar under the longer illumination period is not due simply to increased photosynthesis, since under these conditions the thickened root undergoes shrinkage as a result of dissolution and translocation of storage forms of carbohydrate.

TABLE XVI.—*Reducing sugar in sap of radish exposed to an 18-hour day and to the natural length of day of winter.*

Material.	Reducing sugar in 25 cc. of sap, calculated as dextrose.	
	Plants exposed to an 18-hour day.	Plants exposed to the natural length of day.
	Gm.	*Gm.*
Upper portion of primary stem	0.1192	.....
Lower portion of primary stem	.0185	.....
Leaf stem	.0332	0.0185
Middle section of tuber	.0292	.0193

CONCLUSIONS

The phenomena of photoperiodism as presented in this and preceding papers emphasize the fact that in addition to influencing the fundamental process of photosynthesis—that is, conversion of the chemical elements of carbon dioxide and water into carbohydrate—the duration of the daily light period may definitely control other parallel processes of fundamental importance in plant growth and development. The light period may determine not only the quantity of carbohydrate produced but also the utilization of this material, and it is not possible to explain either the

definite formative effects of the light period or its action on the growth rate on the basis of photosynthesis alone. What these additional processes are has not been determined, but there can be no question as to their fundamental importance. In the present paper data are presented which indicate that the light period in some way profoundly influences acidity relations, the form of the carbohydrate present in the plant and probably the water content of the tissues. Daily periodicity in content of total uncombined acid as affected by light is an outstanding feature of acidity relations in fleshy plants, but in the types of thin-leaved species here dealt with this daily periodicity is of much smaller magnitude. In the present paper the influence of the relative length of day and night on the average level of active acidity in the plant, as measured by the hydrogen-ion concentration of the sap, is considered in some detail. It is shown that growth relations and definite form of expression as controlled by length of day are regularly associated with characteristic acidity relations.

In the case of short-day plants, indeterminate upward elongation of the vegetative stem, which is a characteristic response to a relatively long daily illumination period, is associated with progressive increase in active acidity of the plant, particularly in the region of the growing point. This increase continues till the upper portions of the plant become more acid than the lower portions. On the other hand, exposure to a relatively short daily light period sharply limits increase in stature and quickly initiates flowering and fruiting. Under these conditions a brief transitory period of decreased acidity is followed by only a moderate increase until a level is approached at which flowering is initiated. This level of acidity is much below that characteristic of the advanced vegetative stage under long-day conditions. Under the short-day exposure the upper portions of the plant are less acid than the lower portions. After flowering has been initiated there is progressive increase in acidity in the vegetative parts of the plant. As to the reproductive structures themselves, the embryonic flower bud is relatively low in acidity, while growth of the bud is accompanied by increasing acidity which reaches a maximum in the unfolded blossom. The developing seed, on the other hand, shows a progressive decline in acidity during the period of active growth. Abrupt transfer from a long day to a short day causes a sudden and sharp decrease in acidity in the region of the growing point, which usually occurs about three to five days after the transfer has been made. This drop in acidity, which is believed to indicate definite transition from the vegetative to the flowering condition, is only temporary and is followed by an equally rapid rise to approximately the original level of acidity. These changes in acidity are also observed when flowering is initiated by natural decrease in length of day, but the extent and the sharpness of the changes in acidity are more or less proportional to the amount of the change in duration of the light period. The acidity relations resulting from exposure to the long days of summer also obtain when the short daylight period of winter is prolonged by use of electric light of low intensity.

In the case of long-day plants exposure to a relatively short day tends to inhibit stem elongation, resulting in the leaf-rosette type of development, with or without tuberization. Under these conditions the acidity of the plant remains at a relatively low level. Exposure to a relatively long day more commonly results in elongation of the axis, followed by

flowering. This form of development is associated with general increase in acidity. There appear to be some differences in detail, however, as to the acidity relations in the type of plant represented by summer radish, in which tuberization is a prominent feature, as compared with the type represented by *Rudbeckia bicolor*, in which tuberization ordinarily does not occur. In radish, increase in the duration of the daily light period causes increased acidity in the leaf, and acidity is greater in the upper portion of the stem than in the lower even in the earlier stages of stem elongation. In Rudbeckia, increase in the light period causes decreased acidity in the leaf, and the upper portion of the stem is consistently less acid than the lower portion until after the blossom has unfolded. In both radish and Rudbeckia, however, the acidity of the upper portion of the stem increases progressively both before and after the appearance of flower buds. The acidity relations in the Rudbeckia type of plant when exposed to long days are strikingly similar to those of short-day plants when the latter are exposed to short days. In both instances the acidity of the stem increases moderately prior to the appearance of flower buds, and the reproductive structures themselves increase in acidity as growth proceeds, maximum acidity being found in the unfolded blossom. Moreover, the upper portion of the developing stem is less acid than the lower portion, in contrast with conditions in short-day plants when these are exposed to long days.

There is a group of plants which occupy a position intermediate between the more typical short-day and long-day groups in their responses to differences in the length of day. This group shows some of the features of both short-day and long-day plants. Thus, in *Helianthus annuus* the time of flowering is not materially influenced by the natural range in length of day of spring and summer at Washington, but the stature attained is greatly affected. Increasing the daily light period to 18 hours, moreover, causes considerable delay in flowering. Thus, under the relatively long days of summer this species shows much the same behavior as do short-day plants when they are exposed to short days or long-day plants when exposed to long days. It was found also that the same relationships hold as to acidity of the cell sap. On the other hand, when exposed to an 18-hour illumination period this sunflower approaches in behavior that of short-day plants when exposed to long days. Here, again, the rise in acidity of the sap resulting from the longer light period shows an approach toward the condition of high acidity found in the short-day plants when exposed to long days. This type of plant, therefore, forms a connecting link between the two groups of short-day and long-day plants.

Preliminary studies, which are now being followed up with more extensive observations, indicate that changes in form of the carbohydrate content and in the degree of hydration of the tissues of the plant are among the earliest observable effects of change in the length of day to which the plant is exposed. Transfer of cosmos plants from a long to a short day resulted in a material increase in reducing sugar in the upper portion of the stem within 48 hours after the transfer had been made. Two days later the increase in sugar content was found to be in the form of polysaccharid, and apparently this was accompanied by a slight decrease in water content of the tissues. Twelve days later, after flower buds had appeared, the increased content of sugar was again in the form of monosaccharid. A notable increase in water content of the tissues also had taken place. In Biloxi soy beans exposed to natural length of

day of late summer and fall a slight increase in reducing sugar was found in the sap of the leaf at about the time flower buds were laid down, followed by a decrease at the time open blossoms appeared. A marked increase in reducing sugar and a smaller increase in soluble nitrogen was observed 15 days later, when development of the fruit was rapidly progressing. In summer radish, elongation of the stem resulting from exposure to a long day is associated with an increased content of reducing sugar in the tissues and maximum concentration is found in the upper portion of the stem.

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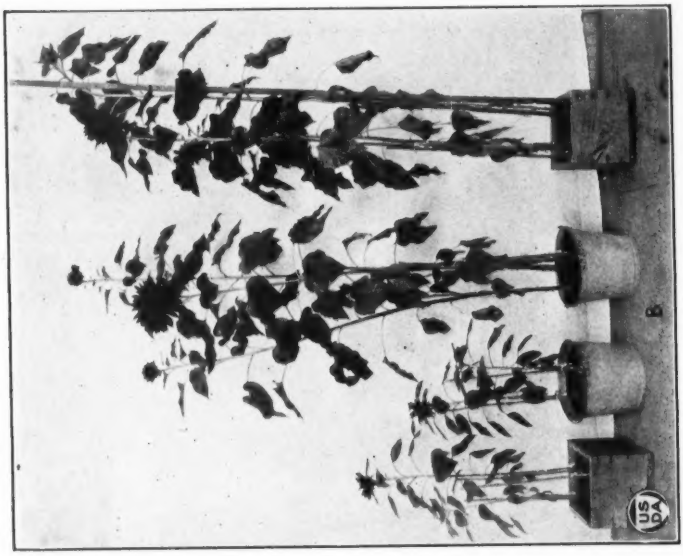
PLATE 1

A.—*Bidens frondosa* L. Plants from seed which germinated December 1. The individual at right was exposed to the natural daylight period of winter supplemented with electric illumination of low intensity from sunset till midnight each day. Individual at left received similar treatment till January 30 after which it was exposed to the natural daylight period only. Shortening the duration of the daily illumination period promptly initiated flowering and open blossoms were seen March 1, the plant having attained a height of 15 inches. When the photograph was made, April 17, the plant at left had ripened its seed and was dead. The plant at right, which remained under the long daily illumination, had attained a height of 6 feet without flowering. This species shows the typical behavior of short-day plants.

B.—Late-flowering cosmos (*C. bipinnatus* Cav.), showing change from the flowering condition back to the vegetative stage as a result of increase in length of the daylight period to which the plant was exposed. This plant, which germinated May 15, was exposed to a 10-hour day till June 13, and thereafter it was exposed to the full daylight period of summer. Flower buds appeared as early as June 3 and flowering had begun by June 19. It is apparent that when photographed, July 20, the new branches developing under long-day conditions were primarily of the vegetative type, though some flower buds were still developing. Though the indeterminate vegetative type of growth is thus restored by increase in length of the daily illumination period, the flowering condition, once it has been established, is completely destroyed only very slowly and with difficulty, if at all.

B

A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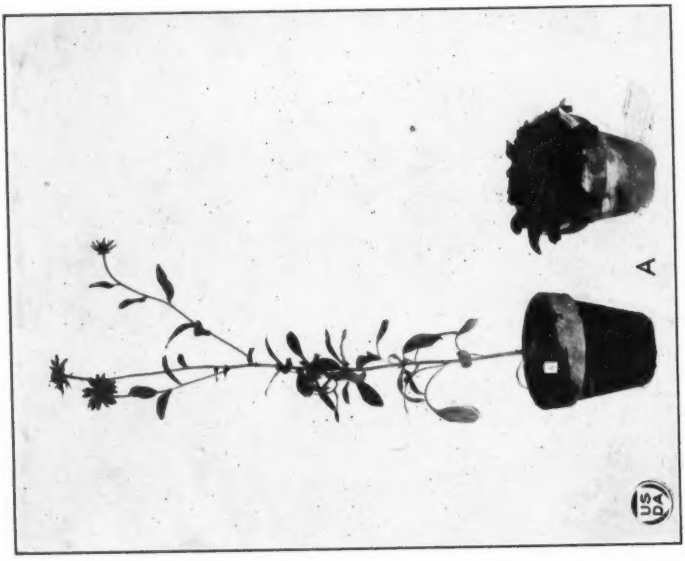
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PLATE 2

A.—*Rudbeck a bicolor* Nutt. Plants from seed sowed December 19. The individual at left was exposed to the natural daylight period of winter supplemented by electric illumination from sunset till midnight. The individual at right received only seven hours of illumination daily. Photographed May 14. The behavior of Rudbeckia is typical of the group of plants which quickly flower in response to relatively long days, and therefore are designated as long-day plants. Ordinarily in this group the height attained is more or less directly proportional to the length of day.

B.—*Helianthus annuus* L. Seedlings which germinated January 23. The larger plants at right were exposed to the natural daylight period of winter and spring, while the smaller plants at left received only seven hours of illumination daily. Photographed April 12. The time of flowering was not affected by the reduced illumination period, but the height attained was markedly decreased. On the other hand, if the illumination period is increased to 18 hours, the time of flowering is delayed. This sunflower shows some of the characteristics of both short-day and long-day plants.

ON THE ANATOMY OF THE SWEET POTATO ROOT, WITH NOTES ON INTERNAL BREAKDOWN

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INTRODUCTION

The fleshy roots of the different varieties of *Ipomoea batatas* Lam. are fusiform, napiform, or irregular-spherical. The surface of the skin appears uniform and smooth in some varieties, and in others irregularly ribbed, because of the presence of vein-like prominences. The secondary roots are arranged in more or less straight vertical rows. Each of these rootlets sits in a shallow depression surrounded by an arch of rough, scar-like tissue, which in shape and texture closely resembles a potato eye. Occasionally the fleshy roots are deeply lobed so that the rows of lateral rootlets lie in longitudinal grooves.

The root nature of these fleshy structures was first shown by Turpin (*6*),[2] who published figures comparing the roots of Ipomoea to the tubers of the Irish potato and the Jerusalem artichoke. Recently Kamerling (*3*) and Tuyihusa (*7*) have taken exception to this view and claimed that these fleshy structures are modified stems. But the validity of this assumption, however ably defended otherwise, becomes untenable when young material is studied. The exarch position of the protoxylem decides, without further argument, in favor of the root-structure theory of these organs.

Since the sweet potato belongs to a group of plants which are distinguished by a peculiar anomalous growth, its anatomical structure has been studied indirectly by a number of investigators, notably by Schmitz (*5*). The only direct contribution to the knowledge of the internal structure of the sweet potato is a short treatise by Miss McCormick (*4*), who also has reviewed all pertinent literature.

THE STRUCTURE OF THE YOUNG ROOT

The young root shows in transverse section several groups of vascular tissue, separated from a thick cortex by an endodermis. (Fig. 1.) The vascular tissue is arranged radially, the xylem and phloem in alternating strands. The number of protoxylem points varies, but most of the roots are pentarch or hexarch. In the region near the growing point, the first protoxylem elements to mature are those farthest away from the center; the development therefore is centripetal. The later maturing protoxylem elements approach each other more and more closely with the increasing age of the region. The protophloem forms small, oval groups lying between the strands of protoxylem and within the circle of the youngest protoxylem element. A parenchymatous sheath, composed of small cells which lack intercellular spaces, intervenes between phloem and

[1] Accepted for publication Nov. 24, 1923.
[2] Reference is made by number (italic) to "Literature cited," p. 166.

xylem. Both xylem and phloem are separated from the endodermis by a single-layered pericycle.

The cortex forms a broad band of tissue composed of large cells with conspicuous intercellular spaces. The peripheral cells are covered by a root epidermis which soon becomes torn and is later replaced by a periderm.

The secondary rootlets take their origin in the pericycle from meristematic cells opposite the protoxylem groups. There are commonly as

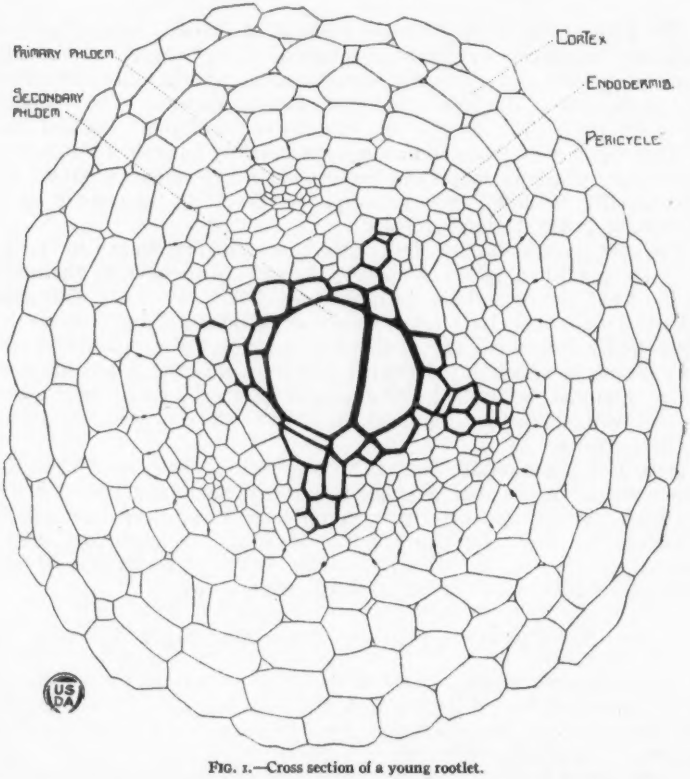

FIG. 1.—Cross section of a young rootlet.

many rows of lateral rootlets as there are protoxylem points. This correlation is well marked in certain overgrown roots, which show five or six prominent ridges and the same number of rows of lateral rootlets in the intervening grooves.

EARLY DIFFERENTIATION AND DEVELOPMENT OF THE ROOT

Even before the protoxylem points are differentiated sufficiently to effect union in the center, growth activity becomes evident in the central parenchyma. Due to this cell increase, the protoxylem groups are forced outward. Meanwhile, one of the most centrally located parenchyma cells has greatly enlarged and matured into a large xylem element.

(Pl. 1 A and fig. 3.) Occasionally an additional small vessel is formed adjacent to the large one.

Following the differentiation of this large "central cell" and the formation of an area of actively growing tissue between the central cell and

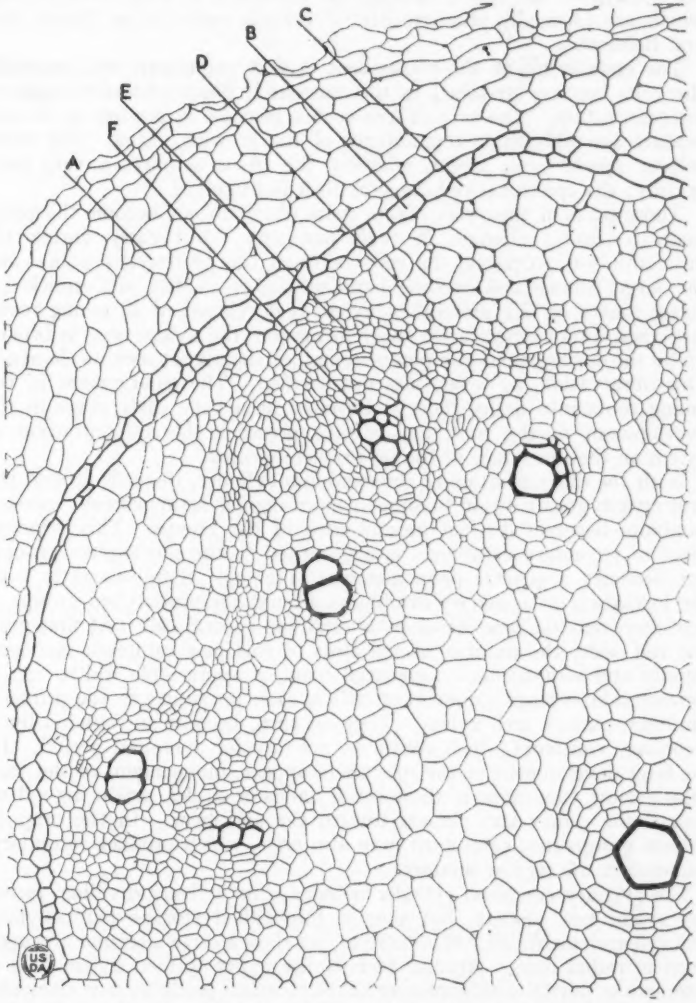

FIG. 2.—Cross section of a young fleshy root. A, cortex; B, endodermis; C, pericycle; D, phloem; E, cambium; F, proto-xylem group. Drawing is a copy of an enlarged photomicrograph.

the protoxylem groups, the differentiation of a cambium takes place. This cambium, lying outside of the xylem and inside of the phloem, gradually forms a complete cylinder. The outline of this cylinder is at first very irregular (fig. 3), partly because of the radial arrangement of the vascular tissue and partly because of local cell increase in the

pericycle opposite the phloem groups. As the root enlarges, cells are produced by the cambium more rapidly in the inner region, and the cambium cylinder becomes symmetrical. Occasionally the irregularities in the activity of the cambium ring continue to be present throughout the growth of the tuber, causing the formation of deep invaginations which are especially characteristic of certain varieties, as Nancy Hall and Belmont.

The endodermis of the young root is very prominent, but gradually the cells become stretched in the tangential plane and show signs of disorganization. The cells of the cortex increase in number to accommodate the widening circumference of the growing organ. The intercellular spaces grow larger, whereby the tissue acquires a very loose texture; the epidermal cells become torn and lignified.

Those parts of the sweet potato roots which do not become thickened undergo similar changes in their ontogeny. But early during the differentiation processes, the cells between the protoxylem points and the large central cell become lignified (fig. 1). Miss McCormick (*4*) states that a certain amount of xylem is developed in all young roots; but when a root enters upon tuber formation, the xylem mass is broken up by the development of a parenchymatous sheath between its elements. This observation, however, was not verified. The enlargement of the young roots into fleshy roots is initiated at a very early stage in the development of the root, and always precedes the differentiation of xylem on the inner face of the protoxylem groups.

With the differentiation of the cambium cylinder, the cells around the protoxylem groups begin to show a more regular arrangement. Soon a cambium becomes distinct around each of the groups. This development of secondary cambiums is not limited to the protoxylem groups, but becomes a general phenomenon. (Fig. 6). Simultaneously with the formation of secondary cambiums around the protoxylem groups, a new meristematic zone develops around the central cell. At first only one cell wide, the number of cell rows of this new cambium increases rapidly and soon forms a tangential band of appreciable width. At a somewhat later stage a number of cells in close proximity to the primary cambium mature into xylem. They, in turn, become surrounded by a secondary cambium which continues activity for a limited period. In the later development of the root there appear, independent of the vascular groups, cambiums in the form of bands or circles, producing xylem toward the center and phloem toward the periphery. In the original phloem groups new cambiums may also arise, which produce secondary elements in the regular manner.

The primary cambium cylinder is meanwhile actively dividing, forming xylem and phloem, but mostly thin-walled storage parenchyma. The xylem elements are few in number, yet show a certain regular arrangement in radial rows. Around each of the newly formed xylem cells a complete or partial cambium may develop, which produces new elements in the regular manner. Often, when the amount of tissue produced by the secondary cambium is considerable, as can best be seen in the region of the large central cell of the young root (Pl. 1, B), a tertiary cambium develops around a number of the secondary elements and increases in the same way as did the group from which it arose. Sections through fleshy roots, which exhibit in mature condition the typical ridged structure previously mentioned, show the ridges traversed by vascular bundles. (Fig. 4.) These bundles originate in the primary cambium cylinder,

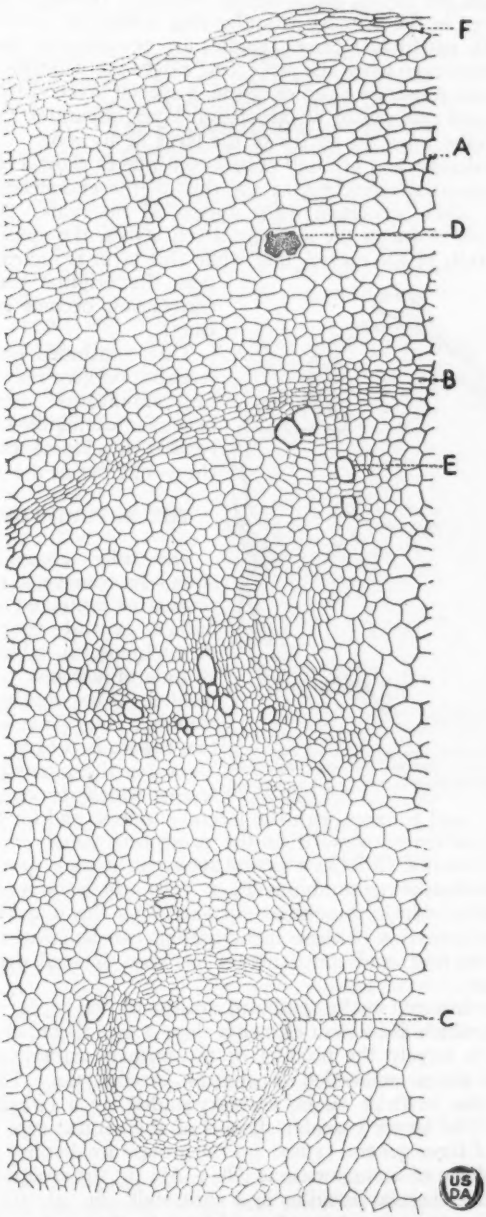

FIG. 3.—Partial cross section of fleshy root about 10 mm. in diameter. A, cortex; B, cambium; C, secondary circular cambium; D, latex cell in cortex; E, secondary xylem, F, periderm.

pass through the cortex, and continue vertically through the veins. The gap produced in the primary vascular ring is filled by a new partial cambium and its products. Small bundles are occasionally seen in the peripheral cortex even when the projecting ridges are wanting. In the case of large veins, a narrow band of tissue, a special layer visible even to the naked eye and not unlike an abscission layer, separates the vein tissue and its bundles from the cortex of the large fleshy root.

The endodermis and primary cortex continue to show a lag in development. Tangential stretching of the cells of the endodermis is still evident, even while anticlinal walls appear at intervals. A periderm is gradually laid down in the superficial cell layers, the epidermis and the peripheral cells of the cortex, and while this layer is increasing in thickness the original cortex and the endodermis become practically extinct.

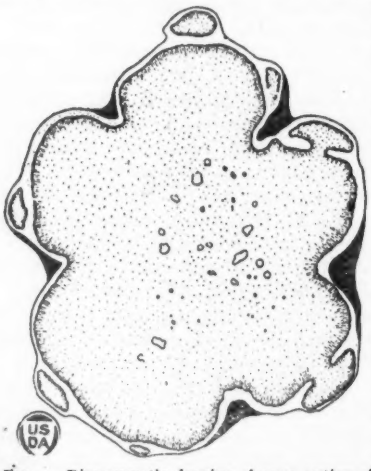

FIG. 4.—Diagrammatic drawing of cross section of mature fleshy root showing the projecting ridges with their vascular tissue.

Thus continued growth and differentiation give rise to a perplexing anomalous structure which becomes intelligible only when the earliest developmental processes in the young organ are followed out.

THE MATURE FLESHY ROOT

The fully grown fleshy root shows in transverse section a narrow cortex protected by a periderm, and a central core of storage parenchyma in which numerous strands of vascular tissue are embedded. The continuity of the cambium ring which separates these two tissues is broken in places, to allow for the passing out of root traces which traverse the fleshy root radially and connect with the lateral rootlets; and for the continuation of cortical traces upward in the projecting veins, where such are present. In certain varieties a second apparent break is produced by narrow invaginations of the cambium (Pl. 3, A, B), which commonly have only a slight vertical depth, sometimes not exceeding the diameter of a root trace. The formation of these invaginations can be related to the early growth of the root, and constitutes a localized delayed development of the cambium.

The tissue internal to the cambium consists of two types: (1) a peripheral zone in which the xylem elements show a typical radial arrangement and which is largely the product of the primary cambium; (2) a core of vascular tissue embedded in storage parenchyma. The individual strands of the vascular tissue are surrounded by a circular or partial cambium. The larger vascular strands of the central core occur in one or another of three general types: (a) the groups are distinct and arranged at the periphery of a narrow circle (Pl. 3, A); (b) the groups are scattered much like the vascular bundles in a corn stalk (fig. 4); (c) the bundles open up, join each other laterally and form in their entirety an intricate network which makes it impossible to trace the individual component

parts throughout the vertical extent of the organ. (Pl. 3, B.) Certain varieties possess characters of all of these groups and can not be readily classified.

However anomalous the grosser morphological structure of *Ipomoea batatas* may be, the tissues and cell forms are similar to those of other dicots. A superficial periderm replaces in protective function the root epidermis. (Pl. 2, D.) The thin-walled cells composing it form parallelopipeds with the shortest diameter in the radial axis of the organ. The outer periderm cells become partly lignified and are progressively sloughed off. The periderm increases in thickness by a phellogen which remains active throughout the season, forming new cells to replace those which are sloughed off as the surface of the fleshy root expands.

The cortex of the mature root is made up almost altogether of secondary tissue. The cells composing it are large starch-filled parenchyma cells, among which at intervals radial rows of sieve-tubes with their companion cells are interspersed. The sieve-tubes are commonly large. The length and course of the cells, however, show great variations in the regions of anomalous growth; it is difficult to find a straight row of any appreciable length. The end walls of the sieve-tubes are normally strictly transverse, but in regions of anomalous growth the end walls may be sharply inclined and even two to three large lateral sieve-plates may be developed. The companion cells are prominent, of small diameter, but in length often equal to their sister cells, the sieve-tubes. Adjacent to the sieve-tubes and, like them, a product of the cambium, are latex tubes. (Fig. 5.) These vessels occur in single vertical rows. The individual segments retain the division walls; in other words, resorption of the cross walls which is observed in other plants does not take place. In young tissue the latex vessels are very prominent and stand out from the surrounding tissue by their larger size and square shape in cross section. The cells have a large nucleus and a white, viscid fluid in which oil drops are suspended. In older tissues the latex tubes are less prominent, since the cells of the tissue surrounding them are of equal size or larger.

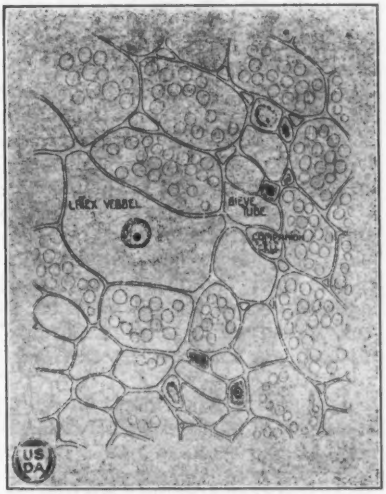

FIG. 5.—Cross section of a radial row of secondary sieve-tubes with their companion cells. Notice the large latex vessel to the left of the row of sieve-tubes.

The cells of the cambium are typical tracheid-like elements and closely resemble those of the common dicots. (Pl. 2, A, B, C, and fig. 6.) In regions of extreme anomalous growth, however, the cells assume various configurations.

All secondary xylem is composed of wide and narrow vessels. These elements have the typical pitted wall structure and communicate with

one another by a single large pore. The lumen of the larger cells becomes filled with tyloses which are most prominent in the mature root.

The storage parenchyma is of two types: (a) Normal bundle parenchyma which, like the xylem and the phloem, is a product of the cambium; (b) interstitial parenchyma—a filler between the groups of bundles. The interstitial parenchyma can be considered the direct progeny of the cells of the parenchymatous sheath of the young rootlet. It forms irregular areas and even broad zones, which are distinguishable from the surrounding tissue by their lighter color. The cells composing it are commonly irregular, elongated, and poor in starch. The cells of the bundle parenchyma are polyhedral and fairly uniform; they are very rich in starch and possess a well developed nucleus.

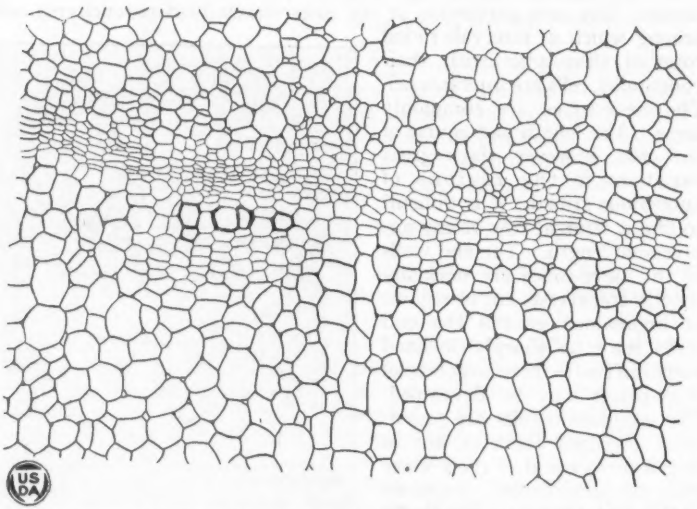

FIG. 6.—Cross section of cambium of large fleshy root. Notice the formation of a secondary cambium to the inside of the group of xylem elements.

TISSUE BREAKDOWN IN STORAGE

Harter and his coworkers (*2*) observed that certain sweet potato roots which had been in storage for a number of weeks would show symptoms of internal breakdown. An examination of the affected areas showed that the cells were partly destroyed and that their starch content was greatly reduced. In general, however, the tissues exhibited no chemical changes which would indicate far-reaching pathological disturbances.

From the study of the structure of the normal tuber, it will be recalled that the groups of vascular tissue are separated by undulating bands or areas of parenchyma. When tubers in the initial stage of breakdown are examined, it becomes evident at once that the first pathological disturbance has taken place in the interstitial parenchyma. The cells of this tissue are large, irregular, and poor in starch; vascular elements and latex tubes are entirely wanting. When breakdown occurs, the cells of the affected region become at first more or less dehydrated; this is indicated by their infiltration with air, giving them a pure white appearance

whereby they stand out strikingly from the surrounding tissue (Pl. 4, A). This parenchyma gradually acquires a spongy texture as the obliteration of cells progresses, leading finally to the formation of small, polyhedral chambers which are lined by the remnants of the destroyed tissue. (Pl. 4, A, B, C, D.)

This process of progressive obliteration begins simultaneously at many points, spreading slowly from the center of the fleshy root along the vertical axis and in extreme cases involving the entire root. During the formation of these cavities, transverse zones of tissue may remain in certain places, forming diaphragms which break the continuity of the longitudinal hollows (Pl. 4, A). The diaphragms appear as thin, translucent membranes from one to several cell layers thick. The walls of the cells are for the most part still cellulose, but some of the elements become lignified. In severely affected fleshy roots the diaphragms also break down, so that long, continuous air passages traverse the entire root.

In advance stages of breakdown, obliteration of the tissue spreads to the parenchyma of the individual bundles, resulting in the denudation and complete isolation of numerous vascular strands.

Except for the lignification of individual cells, the tissue lining the cavities undergoes no change in the composition of its cell walls. Occasionally, however, roots show a pronounced discoloration of the cavities, and a browning and partial lignification of the tissue bordering the hollows. The discoloration may remain more or less confined to the lining of the cavities, or it may spread so as to involve several layers. At such a stage, a section through a root shows a picture not unlike that produced by certain dry rots. The apparent sterility of these cavities and the absence of external injuries, however, suggest no complications resulting from parasitic infections, and the browning of the tissues must be considered, for the present at least, as an advanced stage of the original breakdown.

The hollows which result from the breakdown of the fleshy root tissue resemble in their appearance the lysigenous air spaces found in the stems, leaves, and roots of grasses; sedges, and a host of other plants. The formation of these air passages, according to de Bary (*1*, *p. 216*),

> begins by those cells which do not follow the growth of the tissue surrounding them, becoming at first separated from one another so as to form schizogenous cavities which gradually increase in size. The cells of the tissue thus broken up then gradually lose their protoplasm, dry up and coalesce as flaky masses which are attached to the wall of the cavity. In other cases, the cells first lose their protoplasm, the membranes become apparently thinner, and finally rupture by the extension of the surrounding tissue.

In all these cases the formation of air cavities is a normal phenomenon in the growth and differentiation of the organs of certain plants. The cavities themselves are partly the result of unequal growth of the tissues. Similar agencies are at work when, under abnormal climatic conditions, organs such as Irish potato tubers show extensive internal hollows. These cavities, however, do not develop after the tuber is mature and placed in storage.

While the cavities occurring in the sweet potato have much in common, as far as appearances go, with those resulting in other plants from normal growth processes, and though the mechanism of their formation might be the same, or similar, if we consider typical lysigenous cavities, we have still to determine what factors are responsible for such changes in the

normal metabolism of the resting root. Since breakdown is, to all appearances, correlated with metabolic changes in the resting root, it might be possible to find a satisfactory explanation on a purely mechanical basis by ascertaining the chemical composition of susceptible and resistant fleshy roots and noting the changes which take place under various conditions of storage. From such evidence it may be possible to determine the origin of the trouble, or at least to discover significant correlations which may point to the solution of the problem.

SUMMARY

The edible structure of *Ipomoea batatas* is a thickened root. Its peculiar anomalous structure is due partly to the action of a primary cambium, partly to the development of secondary cambiums and their products.

The cells of the interstitial parenchyma of certain of the varieties break down in storage, causing the formation of polyhedral chambers lined with the cottony débris of the disintegrated tissue.

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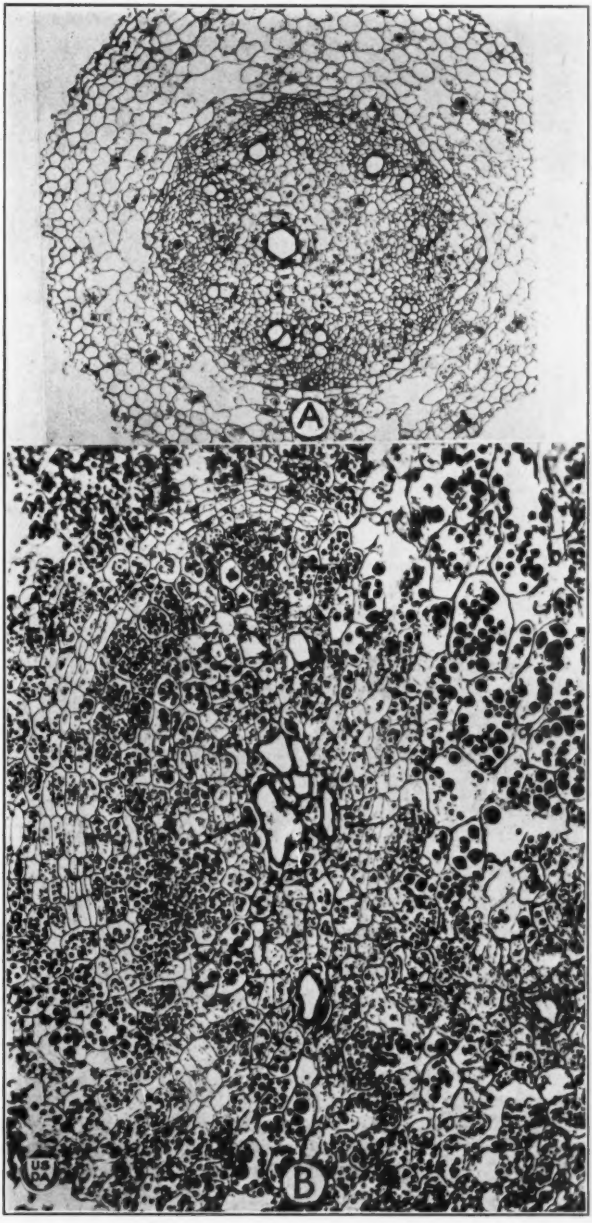
PLATE 1.

A.—Cross section of young, fleshy root (4 mm. diameter). Notice prominent endodermis.

B.—Cross section of large vascular bundle of interior of mature fleshy root. Notice the development of a tertiary cambium around a group of xylem cells in the center of the figure. (Lines are partly redrawn.)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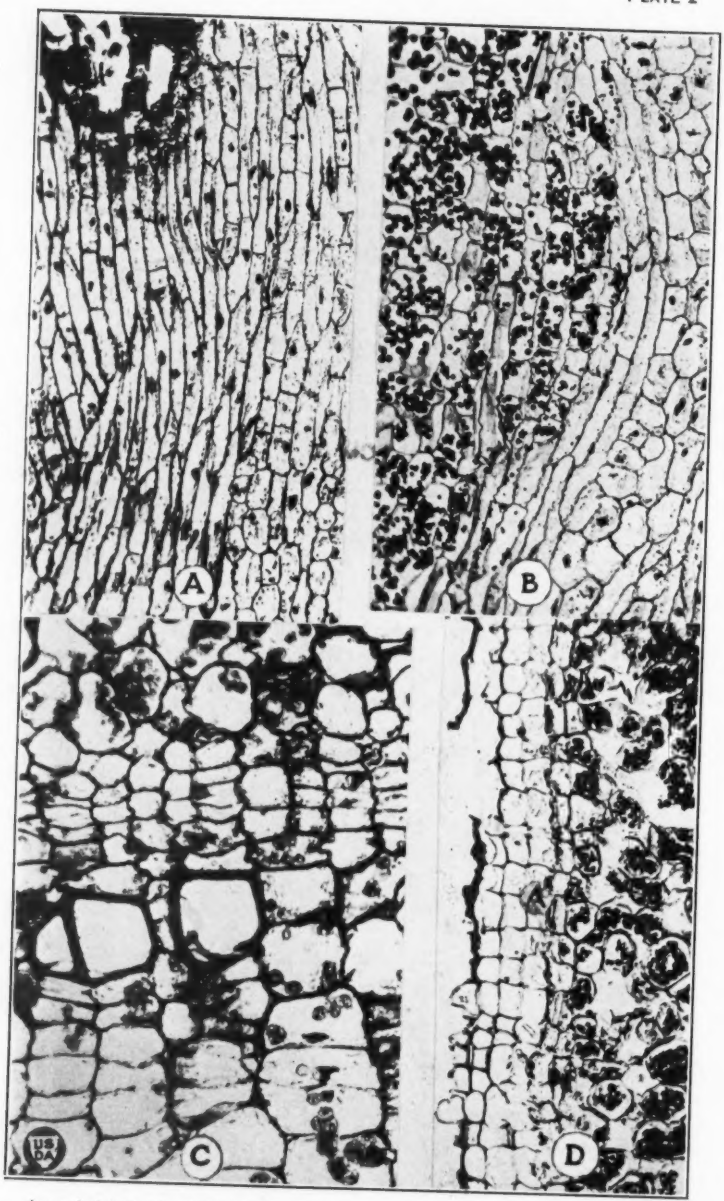
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PLATE 2

A.—Tangential section of cambium of fleshy root.
B.—Radial section through cortex and primary cambium. Notice the abundance of starch in the cells of the cortex.
C.—Cross section through the primary cambium of a mature fleshy root. Notice the formation of a new, partial cambium below the group of xylem cells.
D.—Longitudinal section of the periderm of a young fleshy root.

PLATE 3

A.—Cross section of a mature fleshy root (variety Belmont). Notice that the large center bundles are distinct.

B.—Cross section of a mature fleshy root (variety Nancy Hall). Notice that the larger bundles are united, forming a reticulum.

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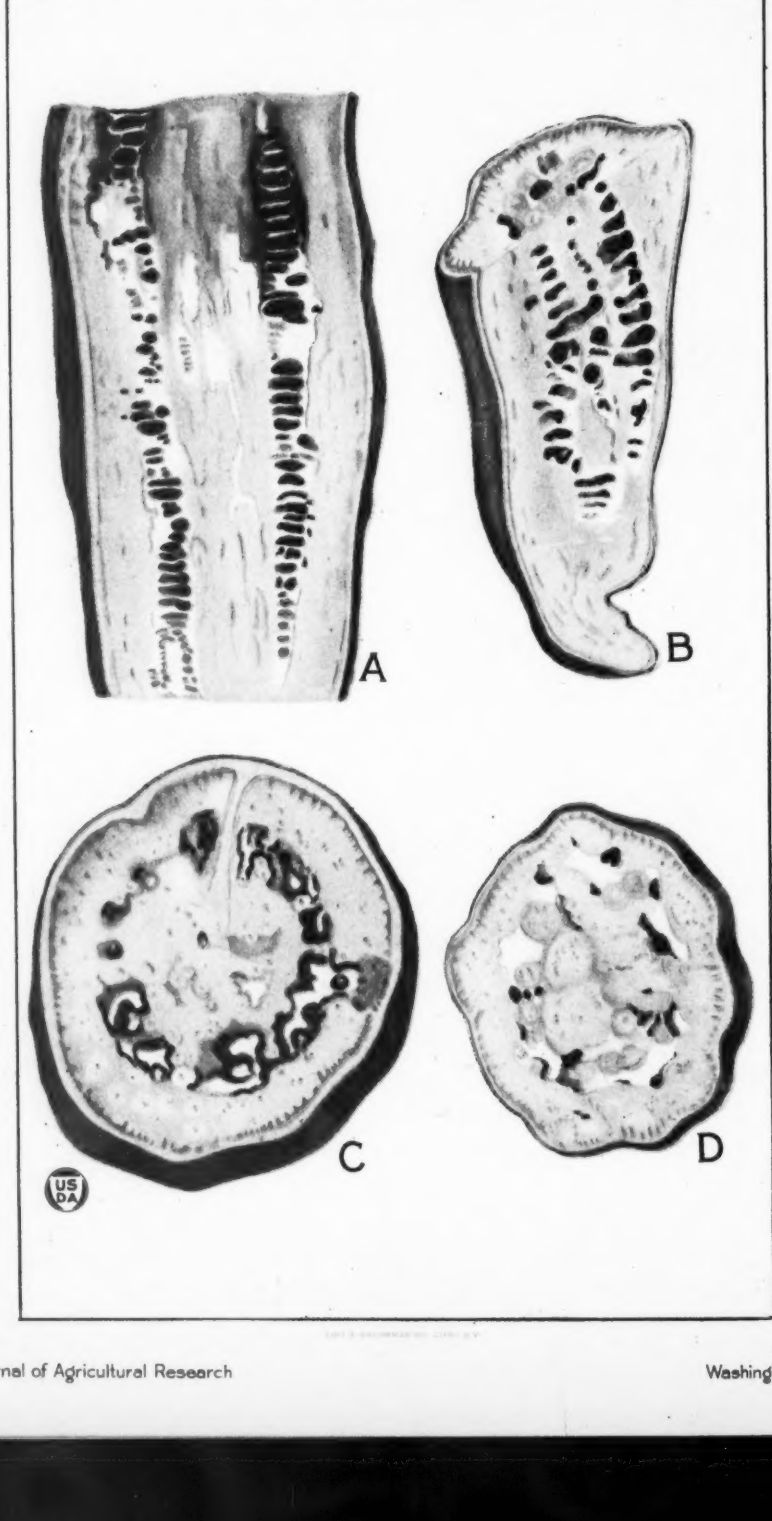
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Plate 4

PLATE 4

A.—Radial cut of fleshy root (variety Key West) showing severe symptoms of internal breakdown. The longitudinal passages are interrupted by diaphragms. The lining of the cavities shows a brown discoloration.

B.—The same as above, except that the breakdown has not yet reached the stage showing brown discoloration. Cavities shaded in drawing.

C.—Cross section of fleshy root shown in A. Notice the denudation of the larger bundles.

D.—Cross section of fleshy root shown in B. This is a typical view exhibited by sweet potatoes affected with internal breakdown.

, D.C.

THE INFLUENCE OF LOW TEMPERATURES AND OF DISINFECTANTS ON THE EGGS OF ASCARIS LUMBRICOIDES[1]

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INFLUENCE OF LOW TEMPERATURES

The results of previous investigations have indicated that the eggs of *Ascaris lumbricoides* are resistant to low temperatures. Wharton (*9*)[3] states that low temperatures retard the development of the eggs but do not kill them; Yoshida (*11*) reports experiments in which the eggs were put on the ground or under a layer of soil and left through the winter months in Osaka, Japan, and their infectivity later proved by their being fed to guinea pigs with resulting infestation in these animals. Martin (*7*) states that the eggs resist freezing and are viable after being in the soil (Nebraska) over winter. In none of the above reports are definite temperature ranges given. Wigdor (*10*) kept eggs of *Toxascaris limbata* in the refrigerator at a temperature of 10° C. and found that their development was retarded but that it ultimately proceeded to the formation of motile embryos.

The present study was made to determine more definitely the possible effect which exposure to low temperatures may have on the normal development of the eggs of *Ascaris lumbricoides* from the pig. To determine the relative resistance of the eggs at different stages in their development, cultures of fresh, of partially developed, and of completely developed eggs (i. e., containing active embryos) were used. Such cultures were exposed to freezing temperatures above 0° F. (= −17.7° C.) and also below 0° F., and their subsequent development after restoration to 24° C. (75° F.) studied. The purpose of these tests was to determine the possibility of such eggs resisting such winter weather conditions as would prevail in pig pens.

TESTS TO DETERMINE THE RESISTANCE OF FRESH ASCARIS LUMBRICOIDES EGGS TO VARYING FREEZING TEMPERATURES

Cultures of eggs were put in Petri dishes, just as squeezed from the uteri of the ascarids, were slightly moistened and put in freezers. The temperature was read two or three times each day.

The following exposures were made (1 day = 24 hours):

For 1 day below zero (−7° to −11° F.).....culture 1.
For 2 days at zero (−2° to +2° F.).....culture 2.
For 5 days:
  Above zero, temperature of 27–28° F.....culture 3.
  Above zero, temperature of 8–11° F.....culture 4.
  Below zero (−0.5° to −11° F.).....culture 5.

[1] Accepted for publication Nov. 19, 1923.
[2] The experimental portion of this study was carried out in the chemical laboratory of Armour & Co. at Chicago, Ill., prior to the author's resignation from that company in October, 1919. This study was a joint project of Armour & Co. and the Federal Bureau of Animal Industry, Mr. Paul Rudnick and Dr. H. B. Raffensperger, respectively, directing the investigation. The writer takes this occasion to acknowledge her indebtedness to these gentlemen for their courteous assistance.
[3] Reference is made by number (italic), to "Literature cited", p. 175.

For 10 days:
Above zero (27.5° to 32° F.).....culture 6.
Below zero (0° to −16° F.).....culture 7.
For 20 days below zero (−14° to +5° F.)[4].....culture 8.
For 30 days below zero (−14° to +5° F.)[4].....culture 9.
For 40 days:
Above zero (10° to 18° F.).....culture 10.
Below zero (−2° to −16° F.).....culture 11.

After being thus exposed to freezing temperatures, the cultures were thawed, covered with potassium bichromate (10 per cent), and kept in an incubator at 24° C. In every case the eggs developed into active embryos, and neither the length of exposure to the cold nor the degree of cold itself seemed to affect the rate of development when the eggs were restored to 24° C. Cleavage appeared in 3 to 5 days, reached the many-celled stage in 6 to 8 days, and the motile embryos developed in 13 to 15 days. Although the low temperature in some cases seemed to break up the protoplasm of the egg so that it lost its normal appearance, this evidently did not interfere with the development, as cells with this appearance decreased in number or disappeared altogether during the incubation.

Although active embryos developed in all the tests, in the last, those for 40 days above and below zero F., the embryos seemed to be short-lived; on the sixth day after motion was first observed only a very few of the many embryos showed any activity. Apparently this prolonged exposure impaired the vitality of the worms, and it seems probable that the infectivity of such worms would be lessened.

TESTS TO DETERMINE THE RESISTANCE TO VARYING FREEZING TEMPERATURES OF PARTIALLY INCUBATED ASCARIS LUMBRICOIDES EGGS—THE EGGS SHOWING VARIOUS STAGES OF CELL DIVISION

Ascaris lumbricoides eggs, developed at 24° C. to various stages of cleavage, were held at freezing temperatures as follows:

For 7 days:
Above zero (8° to 12° F.).....culture A
Below zero (−2° to −16° F.).....culture A′
For 20 days:
Above zero (10° to 16° F.).....culture B
Below zero (−2° to −16° F.).....culture B′

After being kept at these freezing temperatures for the periods of time stated, the cultures were covered with a 2 per cent formalin solution and kept in the incubator (24° C.). All four cultures developed active embryos, and apparently all the eggs, at whatever stage of cleavage when put in the freezer, resumed their development when again incubated at 24° C. That the freezing shortened the life of these embryos was indicated, however, in the second set of tests, those for 20 days above and below zero F. The activity of the developed embryos was quite limited; 24 days after motion was first observed very few embryos could be found which showed any activity.

However, the viability of such embryos, developed from eggs which had been below zero F. for 20 days while in various stages of segmentation, was demonstrated in the following series (C) of tests—in culture F′; the embryonated eggs were fed to a guinea pig and an infection produced in the lungs.

[4] The temperature rose above zero for a brief period in the afternoon on three days.

TESTS TO DETERMINE THE RESISTANCE TO VARYING FREEZING TEMPERATURES OF ASCARIS LUMBRICOIDES EGGS CONTAINING ACTIVE EMBRYOS

Two sets of cultures were kept in freezers, one above zero and one below zero, for the following lengths of time:

For 1 day:
30° to 32° F. ..... culture C.
−13° to −17° F. ..... culture C′.
For 5 days:
30° to 36° F. ..... culture D.
−8° to −17° F. ..... culture D′.
For 10 days:
8° to 16° F. ..... culture E.
−2° to −16° F. ..... culture E′.
For 20 days:
7° to 16° F. ..... culture F.
−6° to −17° F. ..... culture F′.
For 30 days:
12° to 18° F. ..... culture G.
−4° to −14° F. ..... culture G′.

TABLE I.—*Detailed tabulation of tests*

State of egg before freezing.	Culture number.	Temperature to which exposed.	Period of time (days).	Result after restored to 24° C.
Fresh	1	−7° to −11° F.	1	Developed into active embryos.
Do.	2	−2° to +2° F.	2	Do.
Do.	3	27° to 28° F.	5	Do.
Do.	4	8° to 11° F.	5	Do.
Do.	5	−0.5° to −11° F.	5	Do.
Do.	6	27.5° to 32° F.	10	Do.
Do.	7	0° to −16° F.	10	Do.
Do.	8	−14° to +5° F.	20	Do.
Do.	9	−14° to +5° F.	30	Do.
Do.	10	10° to 18° F.	40	Do.
Do.	11	−2° to −16° F.	40	Do.
Partially incubated	A	8° to 12° F.	7	Do.
Do.	A′	−2° to −16° F.	7	Do.
Do.	B	10° to 16° F.	20	Do.
Do.	B′	−2° to −16° F.	20	Do.
Containing active embryos.	C	30° to 32° F.	1	Activity.
Do.	C′	−13° to −17° F.	1	Do.
Do.	D	30° to 36° F.	5	Do.
Do.	D′	−8° to −17° F.	5	Do.
Do.	E	8° to 16° F.	10	Do.
Do.	E′	−2° to −16° F.	10	Do.
Do.	F	7° to 16° F.	20	Do.
Do.	F′	−6° to −17° F.	20	Inactive.
Do.	G	12° to 18° F.	30	Active.
Do.	G′	−4° to −14° F.	30	Inactive.

All five cultures kept *above* zero F. for the stated periods showed active embryos after thawing. Of those kept *below* zero F. the first three (those of the 1-day, the 5-day, and the 10-day periods) showed active embryos. However, that the infectivity of these embryos was diminished was indicated by the fact that neither the culture kept below

zero F. for the 5-day nor the one for the 10-day period produced an infection of the lungs of a guinea pig when fed to it in large amounts. The embryos held for 20 days and for 30 days below zero F. showed no activity after thawing. The former culture (20-day), directly after thawing and when no activity could be seen, was fed to two guinea pigs; no migration of larvae occurred to the lungs of either. However, in this culture there were some eggs that were in the various stages of cleavage; these partially developed eggs formed active embryos when incubated after the freezing period, and these embryos proved infective when fed to a guinea pig. Nevertheless, the evidence showing diminishing vitality with lengthening exposure to low temperatures suggests that there might be a decreasing ability to develop beyond the larval stages to maturity in the normal host, but this is uncertain.

THE INFLUENCE OF DISINFECTANTS

The following series of tests was made as a study of the manner in which the normal development of the eggs of *Ascaris lumbricoides* is affected by exposing them to chemical agents. Experiments by other investigators have shown that ascarid eggs are remarkably resistant. Several different ascarids have been used. Bataillon (*1*), experimenting with eggs of *Ascaris equorum* (=*Ascaris megalocephala*), found that living embryos developed in eggs kept for six months in Fleming's solution and that these embryos remained motile and intact in 50 per cent alcohol 33⅓ per cent acetic acid, and 20 per cent sulphuric acid. Of the dog and cat ascarids, the eggs of *Belascaris cati* (=*Ascaris mystax*) were found by Leuckart (*6, p. 212*) to develop completely in alcohol, chromic acid, and turpentine; Braun (*2, p. 337*) reports a similar resistance to those reagents and to a soda solution by the eggs of *Ascaris canis*, under which name he apparently includes several species. Wigdor (*10*) found that with the eggs of *Toxascaris limbata* nitric acid exhibited the highest ovicidal action of any of the acids, a 5 per cent solution destroying 50 per cent of the eggs, while the efficiency of sulphuric, hydrochloric, acetic, and oxalic acids decreased in the order given. The alkalis (slaked lime, caustic soda, ammonium hydroxid) were devoid of effect, as were also metallic and other salts (corrosive sublimate, copper sulphate, iron sulphate, sodium fluorid, and potassium arsenite), hydrogen peroxid, and a hypochlorite. The embryos were resistant to alcohols up to 70 per cent strength and to formaldehyde up to 35 per cent, but in more concentrated solutions and in volatile oils they either did not develop or were short-lived. The phenols, however, proved the most effective of any of the chemicals used by him, a 1 per cent solution of pure carbolic acid and similar dilute solutions of several commercial preparations entirely preventing the development of the ova.

The eggs of *Ascaris lumbricoides* exhibit a resistance similar to that of the above-mentioned species. With regard to the effect of acids, Galli-Valerio (*3*) found that embryos developed in solutions of sulphuric, hydrochloric, nitric, and acetic acids of 50 per cent or less in strength; more concentrated solutions proved more ovicidal, sulphuric acid being the most effective. Yoshida (*11*) found that embryos develop but soon die in eggs kept in 10–12.5 per cent solutions of sulphuric or acetic or 15–20 per cent hydrochloric acid; in a 1.5 per cent solution of nitric acid they were unharmed. Wharton (*9*) states that the embryos died in eggs kept n 0.5 per cent hydrochloric a cid and in 3 per cent acetic acid. Of the

alkalis, ammonia was tested by Kobayashi (*5*). Yoshida (*11*) had found that eggs were unable to develop in urine, and according to Kobayashi this was interpreted by K. and S. Minagawa as the effect of ammonia formed from the urine. His results showed, however, that eggs survived more than a month in urine and for several days in ammonia (1 to 4 per cent). Salt solutions have little or no effect on the development of *Ascaris lumbricoides* eggs, Galli-Valerio (*3*) having obtained embryos in saturated solutions of copper sulphate, iron sulphate, copper acetate, and 50 per cent antiformin. He states that the eggs are destroyed after four months in pure antiformin. Ransom and Foster (*8*) found that the latter dissolved the shell of the egg but left a thin membrane around the embryo which would protect it from the action of the antiformin for at least five days. According to Kobayashi (*4*) eggs will develop in a saturated solution of sodium chlorid, but the embryos will die when they become mature.

Dilute formalin solutions and also a 10 per cent potassium bichromate solution have proved to be excellent culture media for Ascaris eggs, as they have no ovicidal action and they keep the bacterial count low. According to Kobayashi (*4*) and also Yoshida (*11*), motile embryos will develop in a 10 per cent formalin solution but will not do so in solutions of 20 per cent or higher concentration.

Embryos in eggs kept by Ransom and Foster (*8*) in crude petroleum and in petrolatum were still active after 5 weeks; after 10 weeks those in the petrolatum were dead, but those in the crude petroleum still alive. With regard to the phenols, these writers state that they kept eggs of *Ascaris lumbricoides* containing embryos alive for several hours in carbolic acid. However, reports of Wharton (*9*) and Yoshida (*11*) were to the effect that the embryos do not develop in a 0.5 per cent solution. This is in accord with Wigdor's results, as stated above, with Toxascaris eggs.

The present study was made with phenol solutions in order to determine in greater detail their ovicidal properties and the practicability of their use as disinfectants in the control of Ascaris. It was felt that experiments, to be of practical value, must approximate conditions found in pig pens and that the disinfectant itself and the method of its application must be such as can be used there. Hence relatively pure cultures of eggs totally immersed in solutions of disinfectants would be valuable only to the extent of indicating the possibility of the use of these disinfectants under practical conditions. A disinfectant not proving efficacious under such ideal experimental conditions would certainly not prove so in practice. Therefore experiments were carried out with pure cultures covered with the disinfectant in a beaker and then those concentrations of the disinfectant which had proved ovicidal under these conditions were used for further tests in which conditions were made to simulate those often found in pig pens of sanitary construction: the eggs were well mixed with sawdust and the latter sprayed with the disinfectant.

To determine the relative resistance of *Ascaris lumbricoides* eggs at different stages in their development, cultures of fresh, of partially developed, and of completely developed eggs (i. e., containing active embryos) were used. The cultures were prepared by squeezing the eggs out of the uteri of the worms, covering them with a 2 per cent formalin solution and keeping them in an incubator at a temperature of 20–24° C. until the desired stage of development was reached.

The two disinfectants used were carbolic acid and cresol, the latter in the official compound solution "liquor cresolis compositus." The formula[6] for this is—

Cresol	500 gm.
Linseed oil	300 gm.
Potassium hydroxid	80 gm.
Alcohol	30 mils.
Water, sufficient quantity to make	1,000 gm.

Unless otherwise stated, the exposure of the eggs to the disinfectant was carried out in a beaker, the eggs being covered with a deep layer of the solution and left for the stated time. They were then washed several times in water, put in Petri dishes with 2 per cent formalin and kept at 24° C. in the incubator. In the tests in which the eggs were mixed with sawdust the mixture was spread over the surface of a large flat pan and sprayed with an ordinary atomizer. After the treatment the eggs were recovered by washing the sawdust several times with water and centrifuging the washings; they were then kept in 2 per cent formalin at 24° C.

Two concentrations of each disinfectant were used: 5 per cent and 1 per cent of carbolic acid and 3 per cent and 1 per cent of cresol (6 per cent and 2 per cent of the compound). Both 5 per cent carbolic acid and 3 per cent cresol solution proved very effective in destroying the viability of the fresh eggs and of those in the various stages of cleavage and in destroying the activity of developed embryos. A 3 per cent cresol solution (6 per cent liquor cresolis compositus) accomplished this in 5 hours and a 5 per cent carbolic acid solution in 10 hours. In the tests in which the eggs were mixed with sawdust, thus obtaining conditions which may be found or readily established in pig pens of sanitary construction, it was found that when the mixture was sprayed once with either of the two disinfectants and then allowed to stand 24 hours, the ability of fresh eggs to develop and the activity of developed embryos were not always destroyed. However, if the sawdust mixture was thoroughly raked over and sprayed four times during the day and then allowed to stand over night, the treatment was more effective. Fresh eggs lost all vitality during such treatment with either disinfectant. With 3 per cent cresol all activity of developed embryos was destroyed, and although one active embryo was found directly after such treatment with 5 per cent carbolic acid, on the following day there was no activity. A repetition of such treatment on a second day assured the destruction of all vitality of fresh or developed eggs.

Fresh eggs and eggs at the intermediate stages of development were influenced to about the same degree by the disinfectants, although one test indicates that the further development of the latter is more easily destroyed than that of the former: 3 per cent cresol solution for 2 hours completely stopped the development of the partially incubated eggs, but did not do so with the fresh eggs.

Whereas in the freezing tests it was found that fresh eggs were more resistant to low temperatures than those containing active embryos, there are indications that the resistance of the two sorts of eggs may be reversed in regard to disinfectants: a 5 per cent solution of carbolic acid destroyed the vitality of fresh eggs in 5 hours, but embryos showed activity after such treatment, although their subsequent life was short.

[6] U. S. DISPENSATORY (20th edition), p. 631.

TABLE II.—*Tabulated summary of tests*

State of eggs.	Temperature.	Length of time in disinfectant.	Disinfectant used.			
			5 per cent carbolic acid.	1 per cent carbolic acid.	3 per cent cresol. (6 per cent liquor cresol. compositus).	1 per cent cresol. (2 per cent liquor cresol. compositus).
		Hours.				
Fresh	24° C.	2	A few embryos, but short lived.	Active embryos developed.	A few embryos developed.	Active embryos developed.
Do	do	5	No embryos developed.	do	No embryos developed.	Do.
Do	do	10	do	do	do	
Do	do	15½	do	do		No embryos developed. Retest: Active embryos developed.
Do	do	20	A few embryos but short lived. Retest: No embryos developed.	do	No embryos developed.	No embryos developed.
Do	do	24	(*a*) (Eggs under sawdust, sprayed once): A few embryos developed. (*b*) (Eggs mixed with sawdust, raked and sprayed 4 times): No embryos developed.	do	(*a*) (Eggs under sawdust, sprayed once): No embryos developed. (*b*) (Eggs under sawdust, raked and sprayed 4 times): No embryos developed.	(*a*) No embryos developed. (*b*) (Eggs under sawdust, sprayed once): Active embryos developed.
Do	do	48	(*a*) No embryos developed; (*b*) (Eggs under sawdust, sprayed twice): No embryos developed; (*c*) (Eggs mixed with sawdust, sprayed 8 times): No embroys developed.		(*a*) (Eggs under sawdust, sprayed twice): No embryos developed; (*b*) (Eggs mixed with sawdust, sprayed 8 times): No embryos developed.	(Eggs under sawdust sprayed twice): Active embryos developed.
Do	13° C.	5	Active embryos developed.		No embryos developed.	
In intermediate stages of development.	24° C.	2			do	
Do	do	5			do	
Do	do	15½	No embryos developed.	Active embryos developed.		Active embryos developed.
Do	do	20	do	do		No embryos developed.
Do	do	24	do			
Do	do	40		No embryos developed.		
Containing active embryos.	do	2	Activity		Activity	Activity.
Do	do	5	Slight activity; later none.		No activity	Do.
Do	do	8½	No activity			Do.
Do	do	15½	do			Slight activity; later none.
Do	do	20	do	Activity		No activity.
Do	do	24	(Eggs under sawdust, raked and sprayed 4 times): 1 active embryo found.		(Eggs under sawdust, raked and sprayed 4 times): No activity.	
Do	do	48	(Eggs under sawdust, raked and sprayed 8 times): No activity.		(Eggs under sawdust, raked and sprayed 8 times): No activity.	
Do	5-10° C.	5	Activity		Activity	
Do	do	16	do		do	

Cresol in the compound solution used proved to be more effective than an aqueous solution of carbolic acid; a 3 per cent cresol solution (6 per cent liquor cresolis compositus) had the same results on fresh eggs as a 5 per cent carbolic acid solution and was of greater value in the case of active embryos. A 1 per cent carbolic acid solution was too dilute to destroy the viability of fresh eggs when they were put in it for a 24-hour period; it is therefore not a satisfactory and practical disinfectant. A 1 per cent cresol solution (2 per cent liquor cresolis compositus) proved effective for fresh, partially developed, or fully developed eggs when used for a 20-hour period. In this instance, however, it is clearly shown how important is the distinction between the exposure as carried out with a pure culture in a beaker and, on the other hand, under conditions where the eggs are mixed with sawdust. In the latter case neither a thorough spraying with 1 per cent cresol and subsequent standing of the mixture for 24 hours, or even a repetition of the treatment on the second day destroyed the vitality of the fresh eggs. This weaker solution of cresol is therefore not so practical as the 3 per cent solution.

In regard to the relative efficiency of the disinfectants at 24° C. and at lower temperatures, the following was found to be true: 3 per cent cresol solution (6 per cent liquor cresolis compositus) worked as well at 13 to 15° C. as at 24° C. with fresh eggs (5 hours' exposure destroying their ability to develop), but with developed embryos, although at 24° C., it destroyed their activity in 5 hours, on the other hand, at from 5° to 10° C. it did not do so even in a 16-hour period. With carbolic acid a 5 per cent solution was not so effective on either fresh or developed eggs at refrigerator temperatures as at 24° C.

As to the value of disinfectants in destroying Ascaris eggs in pig pens, it would appear that proper cleaning of the pens is a more useful and more economical method of eliminating the eggs or reducing their numbers to a minimum than the use of disinfectants in the strengths and for the periods of time necessary to kill the eggs, assuming that thorough application of the disinfectants can be secured under practical conditions. The thoroughness of application necessary as indicated by the experiments is such that it would probably not be commonly secured in practice.

CONCLUSIONS

Fresh and partially developed eggs of *Ascaris lumbricoides* show such very great resistance to low temperatures that subsequent development occurred even after the longest periods of exposure: With the fresh eggs a 40-day period at below zero Fahrenheit temperature (−2° to −16° F.) and with the partially developed eggs a 20-day period at the same temperatures. The life of the embryos that developed in these eggs was in both cases relatively short, but in the second case their infectivity for guinea pigs was demonstrated. Developed embryos in *Ascaris lumbricoides* eggs were killed by a 20-day exposure to such temperatures (−6° to −17° F.), but not by a 10-day period at the same temperatures nor by even a 30-day exposure to freezing temperatures above zero (12° to 18° F.). It is therefore evident that while very low temperatures may have a destructive effect upon the vitality of Ascaris eggs, many eggs under natural conditions are likely to survive severe winter weather, and the cold of winter can not be depended upon to destroy the vitality of Ascaris eggs present in pens, pastures, stables, etc. It does, however, diminish their infectivity in the course of time and may aid in controlling

infection by a mechanical action in holding eggs in frozen soil and thus inaccessible to swine.

Both an aqueous 5 per cent carbolic acid solution and 3 per cent cresol in a compound soap solution (U. S. liquor cresolis compositus) destroyed the capability of development in fresh and partially developed eggs and destroyed the activity of developed embryos. This was accomplished in 10 hours by the first disinfectant and in 5 hours by the second, when the eggs were completely immersed in the liquids. Under conditions as nearly analogous as possible to those in sanitary pens in which pigs are kept, the efficacy of the disinfectants was assured if the mixture of eggs and sawdust was thoroughly raked over and sprayed four times on each of two consecutive days. From these results it appears that phenol and cresol disinfectants may prove of value in helping to destroy Ascaris infection in pens and buildings under special conditions. The mechanical action of thorough scrubbing and cleaning and the heat of scalding water which should be used in cleaning are of great importance in the destruction of Ascaris eggs, and the destructive action of chemicals alone should not be expected to replace these measures.

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